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Editorial

ADP-Ribosylation Reactions in Animals, Plants, and Bacteria

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PARP2017, a meeting on ADP-ribosylation reactions, was held in Budapest (17–19 May 2017). The event is held every two years in Europe, alternating with the USA session, held at Cold Spring Harbor Laboratories. ADP-ribosylation is a reversible post-translational modification that has been studied in animals, plants, and bacteria, in both plant and human pathogenic species. ADP-ribosyl transferases (ARTs) are enzymes that add poly-ADP-ribose (PARylation) and mono-ADP-ribose (MARylation) to proteins. In recent years, the topics of PARP/ART proteins have attracted a new wave of interest. In the human genome, there are 15 genes coding for ART proteins, also named PARPs (PARP1–PARP16). The rapid evolution and variability of PARP domains in PARP genes suggests a role of ADP-ribosylation in host-virus conflicts [1]. PARP13 restricts the replication of various families of viruses. PARP13 shows sites of positive selection in the PARP catalytic domain, which has been identified as a target for genetic conflicts with viruses. PARP13 Zn finger directly binds to viral RNA and recruits the exosome for the specific degradation of viral RNA. An evolutionary conservation and divergence among primates and non-mammal species of specific regions of PARP9, 14 and 15 (the Macrodomain containing PARPs), PARP13 (ZAP), and PARP4 involved in the Vault complex [2]. Also, PARP1, 7, 10, and 12 have been shown to play roles in repressing viral replication.

ADP-ribosylation is a reversible post-translational modification (PTM) that is thought to modify very few amino acids, mainly glutamate residues, with mono-ADP-ribose (MAR) or poly-ADP-ribose (PAR). Bacteria toxins, as an exception, are known to modify other residues. For this activity, ADP-ribosyl transferases (ARTs) have been defined as writers [3]. ART enzymes are collectively named MAR- or PAR-writers. ADP-ribosylation exerts allosteric effects on enzymes, thereby controlling their catalytic activity, and can also modify amino acids that are no longer available for other types of PTM, such as phosphorylation or methylation. Moreover, the MAR and PAR regions can be read by multiple protein motifs mediating protein-protein interactions. Recent reviews have deepened our knowledge on the role of enzymes in PARylation and in the sensing and recognition of PAR/MAR by enzyme writers, readers, and erasers [3,4]. PAR/MAR readers are suggested to mediate the functional downstream consequences of ADP-ribosylation. PAR structures can be specifically recognized by proteins harboring a PAR-binding motif (PBM), or a PAR-binding zinc finger (PBZ) motif; as well as by the tryptophan–tryptophan-glutamate (WWE) domain, by the oligonucleotide/oligosaccharide-binding (OB) fold, by the PIN domain in EXO1 nuclease of RAD2 family, and by the Macrodomain of the macroH2A1.1 isoform.

MARylation can be read specifically by ARTD7 (PARP15), harboring two Macrodomains in series. The C-terminal Macrodomain binds to ADP-ribose. ARTD8 (PARP14) is the only human Macrodomain-containing protein that harbors three Macrodomains in series, of which macro2 and macro3 are capable to bind to free ADP-ribose [4] and to MARylated ARTD10 substrates.

The Macrodomains are protein domains that either act as reader modules of ADP-ribose moieties or as erasers, with PAR/MAR hydrolase activity.

Several Macrodomain-containing proteins have been identified; some of them possess only PAR or MAR binding (readers), while other proteins have eraser activity. TARG1, also known as *C6orf130*,

is highly expressed in chronic lymphocytic leukemia (CLL) cells and B cells of CLL patients. MacroD1, also named leukemia-related protein 16 (LRP16), together with MacroD2 and TARG1 has been linked to the release of MAR from MARylated proteins. TARG1 proteins possess hydrolase activity towards MARylated and PARylated proteins, as well as *O*AADPr (O-acetyl ADP ribose) deacetylase activity.

Principal erasers are poly ADP ribose glycohydrolases (PARG), which depolymerize the PAR branches in a cycle of PAR synthesis by PARP1/2, and rapid degradation by PARG. In addition, there are two ADP ribosyl-acceptor hydrolases (ARH), ARH3 and ARH1, that are able to cut the bond between the proximal ADP-ribose moiety and the substrate, fully reversing PARylation and MARylation.

Recently, reviews and novel contributions have shed light on the PARP superfamily [5] in all kingdoms of life [6–8]. In plants, three PARP proteins and several PARP domain proteins have been described [9]. PARP-domain proteins, such as radical-induced cell death 1 (RCD1), have been shown to possess antiviral activity or to be involved in plant response to virus infection. In particular, plants' response to ozone stress or reactive oxygen species (ROS) is dependent on radical cell death 1 (RCD1) and on proteins Similar to RCD One (SRO) to protect the plant from radicals [8,9], and their mutations lead to stress-induced morphogenetic response (SIMR) and developmental defects. Which of the PARP proteins is necessary in plants to protect them from abiotic and biotic stress was a question solved very recently. Using a triple knockout system in Arabidopsis, Rissel and colleagues showed that PARP domain proteins (RCD1, SRO1, and its paralogues) still contribute to protect the plant from these stresses through PAR modification [10].

In plants, a mechanistic understanding of the role of ADP-ribosylation in stress response is still lacking, as well as in plant interaction with bacteria [11]. In the last few months, Feng et al. identified the first set of non-histone plant PARylated proteins, showing that in vivo PARylation of DAWDLE (DDL) is important in plant immunity [12]. DDL is a Forkhead associated (FHA) domain protein, a domain that may bind to PAR regions, is also known to play a role in microRNA biogenesis [13].

In addition to the endogenous plant cell ADP-ribosylation, plant pathogens produce several effectors, among which are ART writers, that disrupt the signaling pathway, leading to plant immunity. For instance, *Pseudomonas syringae* HopU1, a C-type ART with mono-ART activity, HopF2, a D-type ART, and HopM1 are counted among these effectors.

The race between hosts and viruses for the cell machinery involved in replication and infection is never ending. Within stress-associated RNA granules, human viruses during infection subvert the intracellular machinery to the scope of replication [14]. Five cytoplasmic PARPs are involved in stress granule regulation. PARP13/ZAP is a CCCH-type Zinc finger PARP with antiviral activity [15,16]. During the inflammation response, PARP16/ARTD15, involved in stress granule formation, regulates the post-translational modification of importin-α1 (kariopherin- α1), and its ADP ribosylation leads to the formation of stress granules [17]. Various viral species have evolved mechanisms to control the proteins involved in stress granule formation, and to block innate immunity. Macrodomain is a conserved protein fold found in several plus-strand RNA((+)ssRNA) viruses, including alphaviruses and coronaviruses. Viral Macrodomain proteins bind to the small molecule ADP-ribose to counteract the ADP-ribosylation signals in host defence against viruses. The first function assigned to these viral Macrodomains was the processing of ADP-ribose-1″-phosphate, a side product of cellular pre-tRNA splicing, which thus affected the metabolism of ADP-ribose derivatives [18,19]. Moreover, some viral Macrodomains bind to free ADP-ribose, free PAR chains, and PARylated ARTD1 [20], suggesting an involvement in ADP-ribosylation processes. Prototype MacroD-type macrodomains also hydrolyze derivative linkages on the distal ribose ring. The non-structural protein 3 (nsp3) Macrodomain of Sindbis virus (SINV) is a virulence factor important for viral replication. Mutation of the Macrodomain affecting ADP-ribose binding resulted in impaired virus replication as well as viral RNA amplification, and showed reduced neuro-virulence in mice [21]. In addition to the nsp3 Macrodomain of SINV, the nsp3 Macrodomain of murine coronavirus MHV-A59 has been linked to neuro-virulence, and is essential for virus-induced encephalitis [22]. Furthermore, an ADP-ribose-1′-phosphate phosphatase

mutant of the nsp3 Macrodomain of murine coronavirus MHV-A59 showed reduced replication in the liver and impaired development of liver pathology by inhibiting the expression of inflammatory cytokines [23]. The viral nsp3 Macrodomain of Severe Acute Respiratory Syndrome (SARS) coronavirus has been demonstrated to modulate and to facilitate escape from the immune response [24]. Again, a Macrodomain mutant that interferes with ADP-ribose-1′-phosphate phosphatase activity showed increased sensitivity to the IFNα-mediated antiviral response [25]. These findings imply that mutations in viral Macrodomains are important for virus pathology. Chikungunya virus (CHIKV), an Old World alphavirus transmitted to humans by mosquito pricks, possesses the nsP3 Macrodomain, critical for virus replication and virulence: nsP3 is able to hydrolyze ADP-ribose groups from mono(ADP-ribosyl)ated aspartate and glutamate residues, but not from lysines [26]. Thanks to these findings, Robert Lyle McPherson, of Dr. Anthony Leung's team, at Johns Hopkins University in Baltimore, won the best poster award at the PARP2017 meeting; the award was offered by this journal to sponsor the research on PARP/ARTs.

Proteomics approaches have been attempted by various groups in order to study protein ADP ribosylation, especially amino acid residues and sites of ADP ribosylation modifications [27].

The PARP2017 meeting was a great opportunity to discuss and meet with the pioneers of ADP ribosylation studies. In particular, Dr. Alexandre Bürkle, Dean of Konstanz University, gave an extraordinary presentation on the past, present, and future of PARP studies. Dr. Lüscher, a wonderful person and a cheerful and motivating scientist, was actively involved in all the sessions. Dr. Bay was a kind and attentive chairman, and hosted the meeting in the name of the Hungarian Academy of Science, together with the PARP scientific committee. Dr. Hottiger took the stage to present the extraordinary findings obtained by the immunoseparation of PARylated proteins using a mutant Macrodomain [28–30]. The most exciting finding in recent months was the ADP ribosylation at serine residues [31], in addition to lysine being found to serve as an ADP-ribose acceptor site.

The serine modification was found to be possible thanks to the interaction of ART1 with histone PARylation factor 1 (HPF1) [32]. The proteins involved in ADP ribosylation have been included in the database http://ADPriboDB.leunglab.org, curated by the Leung lab [33].

Also, ARH3, considered an eraser enzyme, was presented as a new MAR eraser at serine residues. In particular, the presentations by Drs. Krastev, Nathubai, and Ranes, of the Institute of Cancer Research in London and Bath University, showed how applied science can join basic studies, especially in the development of tools such as PAR/MAR genetic screens and biosensors. Lastly, Dr. Ivan Ahel presented the toxin-antitoxin DarTG system in *E. coli* that controls ADP-ribosylation of DNA during environmental stresses.

Finally, these new advancements offer a timely implementation of the knowledge on ADP ribosylation reactions, with an update on these topics, recently described in two reviews [34,35].

In conclusion, the opportunities of a new generation of young skilled researchers with open minds will help to produce new insights and disclose new findings in the field of PAR- and MARylation. There are still challenges in understanding the ART roles and the many functions in replication, transcription, epigenetic control, and intracellular signaling in all the kingdoms of life, demonstrating that scientific interest in PAR/MAR signaling will never come to an end.

Conflicts of Interest: The author declares no conflict of interest.

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