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Editorial

Emerging Concepts on the Role of ADP-Ribosylation

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Abstract: NAD^+ has emerged as a crucial element in both bioenergetic and signaling pathways, since it acts as a key regulator of cellular and organism homeostasis. NAD^+ is a coenzyme in redox reactions, a donor of adenosine diphosphate-ribose (ADPr) moieties in ADP-ribosylation reactions, and a substrate for sirtuins, a group of histone deacetylase enzymes that use NAD^+ to remove acetyl groups from proteins. NAD^+ is also a precursor of cyclic ADP-ribose, a second messenger in the release and signaling of Ca^{++}, and of diadenosine tetraphosphate (Ap4A) and oligoadenylates (oligo2′-5′A)—two immune response-activating compounds. In the biological systems considered in this review, NAD^+ is mostly consumed in ADP-ribose (ADPr) transfer reactions. In this review, the roles of these chemical products are discussed in biological systems, such as in animals, plants, fungi and bacteria. In the review, ADP-ribosylating enzymes are introduced, as well as the importance to restore the NAD^+ pools in these systems. Finally, a special attention is presently focused on viral macrodomains, aimed to develop inhibitors to improve the immune response to viruses.

Keywords: ADP Ribosyl Transferases (ARTs); post-translational modification (PTM); Macrodomain; ADP ribose glycohydrolases (ARH); ADP ribose (ADPr); Nicotinamide adenine dinucleotide (NAD^+); nicotinamide (Nam); m-iodobenzylguanidine (MIBG)

Introduction

NAD^+ is a substrate used by enzymes involved in post-translational modifications; in particular, this editorial will focus on ADP ribosylation reactions, producing mono ADP ribose (MAR) and poly ADP ribose (PAR) moieties bound to proteins and nucleic acids [1–3]. The intracellular concentration of NAD, its degradation to nicotinamide and its rapid, temporary unavailability (especially into localized energy compartments) are of fundamental importance to cell health or death.

When the reaction involves a single monomer attached to proteins, it is named mono ADP ribosylation (MARylation, MAR), while the attachment of a complex, branched polymer to proteins is named poly ADP ribosylation (PARylation, PAR).

The amino acids being modified during protein ADP-ribosylation are various, the attachment may occur on carboxylic groups, such as on Aspartate (D) and Glutamate (E), on amino or guanidino groups, such as in Lysine (K) and Arginine (R), thiol group in Cysteine (C) (non-enzymatic attachment), and on hydroxyl groups as in Serine (S). Therefore, various and different post-translational modifications may modify the same acceptor amino acid, precluding its modification by another type of enzyme, or may impede the access to a neighbor site by hindrance, or by repulsing charges.

ADP-ribosyl transferases (ARTs) are described as writers [4], either MAR- or PAR- writers. ADP-ribosylation exerts allosteric effects on enzymes, thereby controlling their catalytic activity, as well as taking together proteins that need to be in tight proximity to form protein complexes activating specific pathways, such as immune response, DNA damage response, and transcription activation. The post-translational modification of macromolecules requires that the reaction be reversible, coupling enzymes that catalyze the modification (ARTs, writers) with the erasers enzymes that reverse the

modification, poly ADPR-glycohydrolases (PARG), and ADP ribosyl-acceptor hydrolases (ARH). PARG depolymerizes the PAR branches in a cycle of PAR synthesis by PARP1/2, and rapid degradation by PARG. In addition, ARH3 and ARH1 are able to cut the bond between the proximal ADP-ribose and the amino acid [1].

The group of ADPR modification enzymes, in addition to writers and erasers, include reader proteins: binding to MAR and PAR regions occurs by Macrodomain (recognising the terminal ADP ribose), by WWE (tryptophan/glutamate) and PAR Zinc finger (PBZ) domains (binding to ADP ribose-ADP ribose junctions), by PAR Binding Motif (PBM), by Forkhead-associated (FHA) domains (recognising the ribose-diphosphate-ribose adenine region), by oligonucleotide binding fold (OB fold), by BRCA1 C-Terminal region (BRCT) in the BRCA1 antioncogene, and PilT N-terminal (PIN) domain in PIN and Exo1 nucleases, by RNA recognition Motif (RRM), by serine arginine repeats and lysine arginine repeats (SR/KR) and RGG repeats in RNA binding proteins,: docking to mono and poly ADP ribose regions.

Several Macrodomain-containing proteins have been identified; some of them possess only PAR or MAR binding (readers), while other proteins have eraser activity. Macrodomains carrying glycohydrolase activity can release the MAR/PAR from the modified amino acid of the protein target.

Viral infection leads to the formation of the stress granule, but viruses are able to exploit this cellular response for their translation and replication. Several plus-strand RNA (+ssRNA) viruses, including alphaviruses and coronaviruses contain proteins with the macrodomain fold. Viral Macrodomain proteins bind to ADP-ribose and counteract ADP-ribosylation signals in host defence against viruses, altering the stress granule formation, and metabolism of ADP-ribose derivatives [2]. The non-structural protein 3 (nsp3) Macrodomain of Sindbis virus (SINV) is a virulence factor important for viral replication. The nsp3 Macrodomain of murine coronavirus, of Severe Acute Respiratory Syndrome (SARS) coronavirus and of Chikungunya virus (CHIKV) are critical for virus replication and virulence.

In addition, researchers found that PARP12 and PARP14 are host cell ADP-ribosylating enzymes important for the attenuation of viruses encoding the Macrodomain protein [4]. PARP2019, one of the largest biannual meetings on ADP-ribosylation reactions, was held in Budapest from 20–23 May 2019, focusing on "New avenues in basic and translational PARP research". In 2017, the journal Challenges and MDPI contributed to the organization of PARP2017, also held in Budapest, with the best poster award to Robert Lyle McPherson, of Dr. Anthony Leung's team, at Johns Hopkins University in Baltimore [2]. Thanks to the support of Leung Lab, the team produced recently a new publication on the potential to treat virus infection with inhibitors of viral macrodomain [5].

Among coronaviruses, in addition to Severe Acute Respiratory Syndrome (SARS) and 2019-nCOV (also named SARS-CoV2 or COVID19), CoVs-229E, CoVs-NL63, CoVs OC43 and HCoV-HKU1 can also cause respiratory diseases in humans. The first two of these human coronaviruses are α-coronaviruses, while the other two are β-coronaviruses. HCoV-229E and HCoV-OC43 were isolated nearly 50 years ago, while HCoV-NL63 and HCoV-HKU1 have only recently been identified following the SARS-CoV outbreak [6,7].

This year, during PARP2019 preparation and beyond, two Special Issues were launched and concluded. the first one, on the journal Biochemical Pharmacology, on the emergence and the need of new ART inhibitors. Seventeen papers contributed to the Special Issue [8], ranging from reviews on the importance of inhibition of ARTD and ARTC enzymes, the reaction of PAR and formation, to PAR degradation by PARGs and the role of inhibitors in anticancer therapies. Contributions were grouped into molecular biology and biochemistry/molecular biology, in cell biology, pathophysiology, and in treatment of cancers, with a focus on ART inhibitors and their association with other types of inhibitors.

The second Special Issue following PARP2019 was published on Cancers (MDPI), titled "PARPs, PAR and NAD Metabolism and Their Inhibitors in Cancer", (https://www.mdpi.com/journal/cancers/special_issues/parp2019).

This year, a Cold Spring Harbor Laboratory (CSHL) meeting series will be held from April 1–4, on The PARP family and ADP-ribosylation, organized by L. Kraus, S. Smith and A. Ladurner (https:

//meetings.cshl.edu/meetings.aspx?meet=parp&year=20). As of February 2020 [9], the call has already produced eight articles in advanced online status, published by Genes and Development. Among these reviews, "Interplay between compartmentalized NAD+ synthesis and consumption" reviews on the activity of PARPs and other NAD+ consuming enzymes, regulated in a compartmentalized manner. In the effort to have availability of NAD+ for metabolic processes, different subcellular pools of NAD+ are regulated, and free NAD+ levels control signaling by PARPs and redox metabolism [10]. The review provides a clue on the way cells are able to overcome stressful conditions in health and disease, when provided with a surplus of NAD+ [3].

Lastly, the Japanese Biochemical Society meeting will hold a PARP/ART symposium titled "Mono- and poly (ADP-ribosylation) pathways for diverse biological regulation", during the annual meeting, to be held on 14–16 September 2020, in Yokohama, Tokyo.

There is an increasing demand for new tools to study the biological activity of these enzymes and, in particular, to find new inhibitors with better specificity and selectivity with respect to m-iodobenzylguanidine (MIBG), a very broad inhibitor of diverse enzymes.

For instance, using fluorophore 4-(trifluoromethyl) umbelliferone (TFMU) coupled to inosine diphosphate ribose (IDPr), Drown and colleagues found that ARH3 is inhibited inside cells by the metabolite ADP-ribosyl arginine, a product of the activity of cholera toxin ART [11].

There is a great potential for substrates of viral nsP3 macrodomains to be used to develop non-cleavable ADP-ribosylated peptides able to inhibit coronaviruses in vivo. There is great hope that new ART inhibitors will provide new tools for the treatment of emergent viral diseases such as 2019-nCOV.

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

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