Posttranslational modification

The beauty of ADP-ribosylation: versatility in every link and organelle

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ADPr is a crucial modification that regulates various cellular processes. Its complexity stems from the diverse chemical linkages it can form, including connections to various amino acids and nucleic acid components. ADPr can manifest as single ADP-ribose unit or as chains and can also form hybrid modifications. Here we focus on the remarkable versatility of ADPr and the distinct subcellular localization of its writers, which enable it to regulate essential processes in a spatio-temporal manner.

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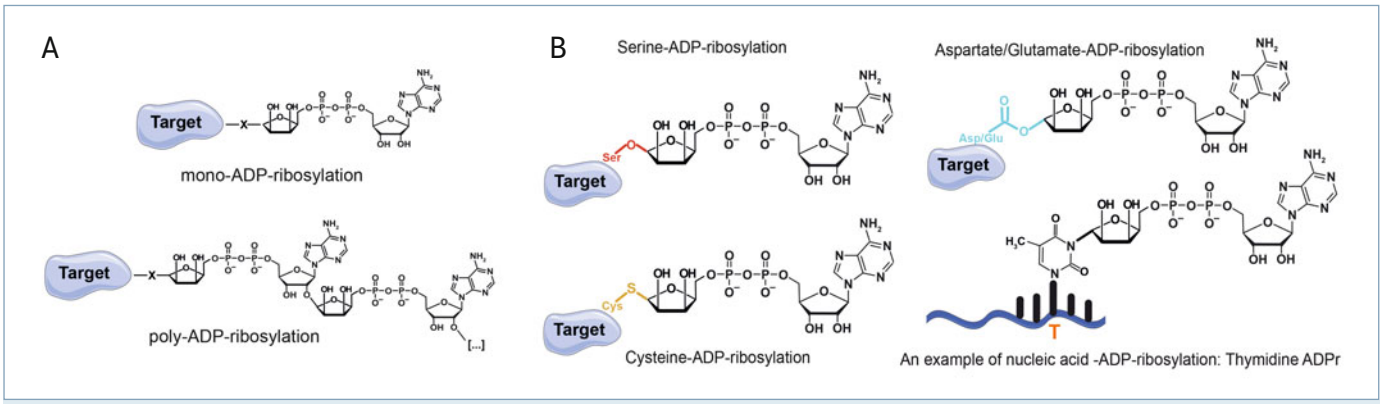
ADP-ribosylation (ADPr) is a fascinating and highly intricate posttranslational modification (PTM) that is widespreadly used for cellular signaling by organisms across all kingdoms of life and some viruses [1]. It is established through the transfer of ADP-ribose units from β-nicotinamide adenine dinucleotide (β-NAD$^+$), a molecule better known for its role in metabolism [2]. Unlike many other PTMs, ADPr is nucleotide-based, consisting of a ribose, two phosphate groups, and an adenine. This unique composition makes ADPr inherently complex to study and has led to some initial misinterpretations and technical challenges, followed by surprising discoveries throughout its history. For example, early research in the 1960s initially misidentified poly-ADP-ribose (PAR) as polyadenylation during studies on RNA polymerase activity. However, further analysis quickly revealed the true identity of the polymer as poly-ADPr, marking the beginning of a new field [3].

ADPr is mediated by a diverse group of enzymes known as ADP-ribosyltransferases (ARTs), which include the diphtheria toxin-like ARTs (ARTDs), commonly known as "PARPs", as an abbreviation of their historical name "poly-ADP-ribose-polymerases". The PARP family comprises at least 17 intracellular enzymes, all characterized by the presence of the distinctive "PARP signature" motif [4].

PARP enzymes catalyze the addition of ADP-ribose to their targets in two distinct forms: mono-ADP-ribosylation (MARylation) or poly-ADP-ribosylation (PARylation) (**Fig. 1A**). In MARylation, a single ADP-ribose unit is covalently attached to target proteins, whereas PARylation involves the synthesis of extended poly-ADP-ribose chains, characterized by a ribose(1″→2′)ribose-phosphate backbone and, in rare cases, branch points with ribose(1″→2″)ribose structures.

For a long time, the field was focused almost exclusively on PARylation, largely because early studies identified PAR as the primary product of PARP activity. Hence, the term "PARP" encompasses all ARTD family members, regardless of their ability to catalyze PARylation. This bias stemmed from technical limitations: early detection methods, such as ADPr antibodies, were highly sensitive to the long polymeric chains of PAR but were unable to detect MAR. As a result, studying the activity of MARylating PARPs was lagging behind, and PARPs such as PARP1, PARP2, and the tankyrases (PARP5a and PARP5b) were thought to only catalyze PARylation.



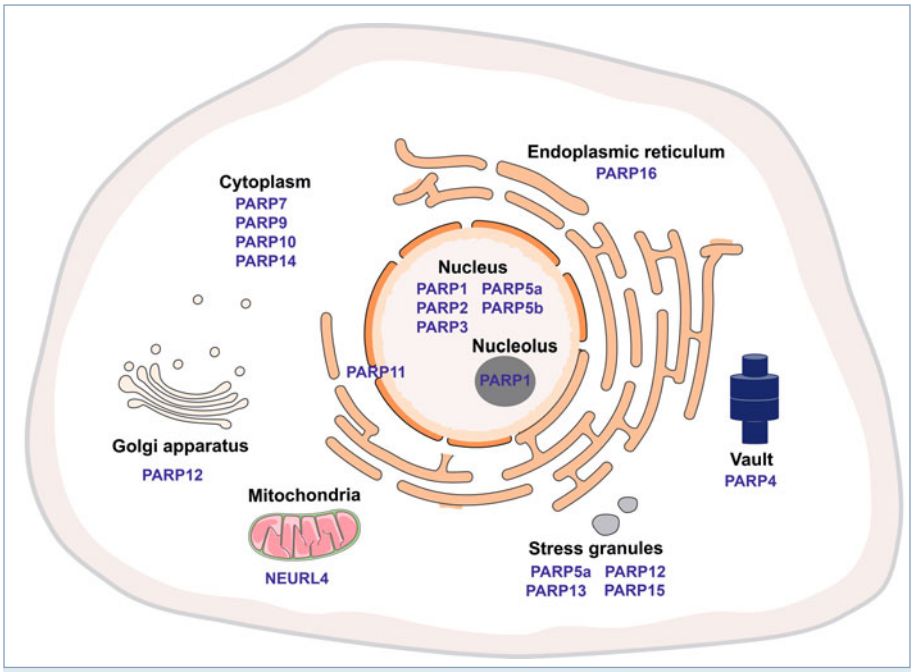
▲ **Fig. 1:** Schematic representation of ADPr linkage versatility. **A**, Mono-ADP-ribosylation (MARylation): a single ADP-ribose unit is covalently attached to target proteins (upper quadrant) and poly-ADP-ribosylation (PARylation): extended poly-ADP-ribose chains, characterized by a ribose(1″→2′)ribose-phosphate backbone are attached to target proteins (lower quadrant). **B**, schematic representation of target residues of ADPr and their linkage, such as serines, cysteines, glutamate and aspartate amino-acids, linked to ADPr via *O*-, *S*-, or *N*-glycosidic bonds, and nucleic acids. T= thymidine.

However, the advent of more sensitive tools, including antibodies capable of detecting MARylation [5], has revolutionized the field. It is now evident that most PARP enzymes primarily catalyze MARylation rather than PARylation. Even PARPs historically associated with PARylation, such as PARP1 and PARP2, were shown to perform MARylation as well. These findings shifted the paradigm: mono-ADPr appears to be the predominant activity of PARP enzymes, while poly-ADPr is the exception. Nevertheless, both are distinct and extremely important signaling events in the cells.

In addition to their ability to catalyze MAR or PAR, PARPs exhibit remarkable versatility in their choice of target residues. These include acidic amino acids such as glutamate and aspartate, thiol-containing cysteines, and hydroxyl-bearing serines, among others (**Fig. 1B**, [6]). Beyond proteins, PARPs can also modify nucleic acid termini and bases [7, 8].

Furthermore, recent studies have shown that the target preference of some transferases can be altered depending on the cellular context. For example, while PARP1 and PARP2 primarily modify acidic residues via ester-type O-glycosidic linkages *in vitro*, they target serine residues through an ether-type O-glycosidic linkage in cells in response to DNA damage [9]. This change in specificity is mediated by the interaction with histone PARylation factor 1 (HPF1) [10].

The wide range of specificity with diverse linkage types has presented an additional technical difficulty for the field, since these linkages can be difficult to detect under widely used protein biology and proteomics workflows. For example, serine-linked ADPr (O-glycosidic linkage) is stable across a broad range of temperatures and pH levels but is highly labile during traditional mass spectrometry fragmentation techniques [11]. Conversely, aspartate- and glutamate-linked ADPr (ester bonds) are stable in mass spectrometry but prone to hydrolysis under alkaline conditions commonly used during proteomics workflows [12]. These technical challenges initially obscured the existence of certain ADPr linkages, leading to debates over their biological relevance. Thanks to optimized protocols and the development of specialized antibodies, researchers can now detect and study ADPr in much of its complexity – though the full picture is still unfolding.



▲ **Fig. 2:** Subcellular localization of PARPs. PARP1, PARP2 and PARP3 are primarily nuclear enzymes. Additionally, some PARPs such as PARP7, PARP9, PARP10, and PARP14, can translocate to the nucleus under specific conditions. Curiously, PARP11 localizes to the nuclear periphery. In the cytoplasm, PARP5a, PARP13, and PARP15 colocalize with stress granules, as well as PARP12, that can transition from the Golgi apparatus to stress granules. PARP16, the only known membrane-associated PARP in humans, is localized to the endoplasmic reticulum membrane. Moreover, PARP4 is associated with vault particles, and the PARP-like protein NEURL4 has recently been implicated in mitochondrial functions. Figure adapted from [4].

Spatiotemporal regulation of PARPs in key biological processes

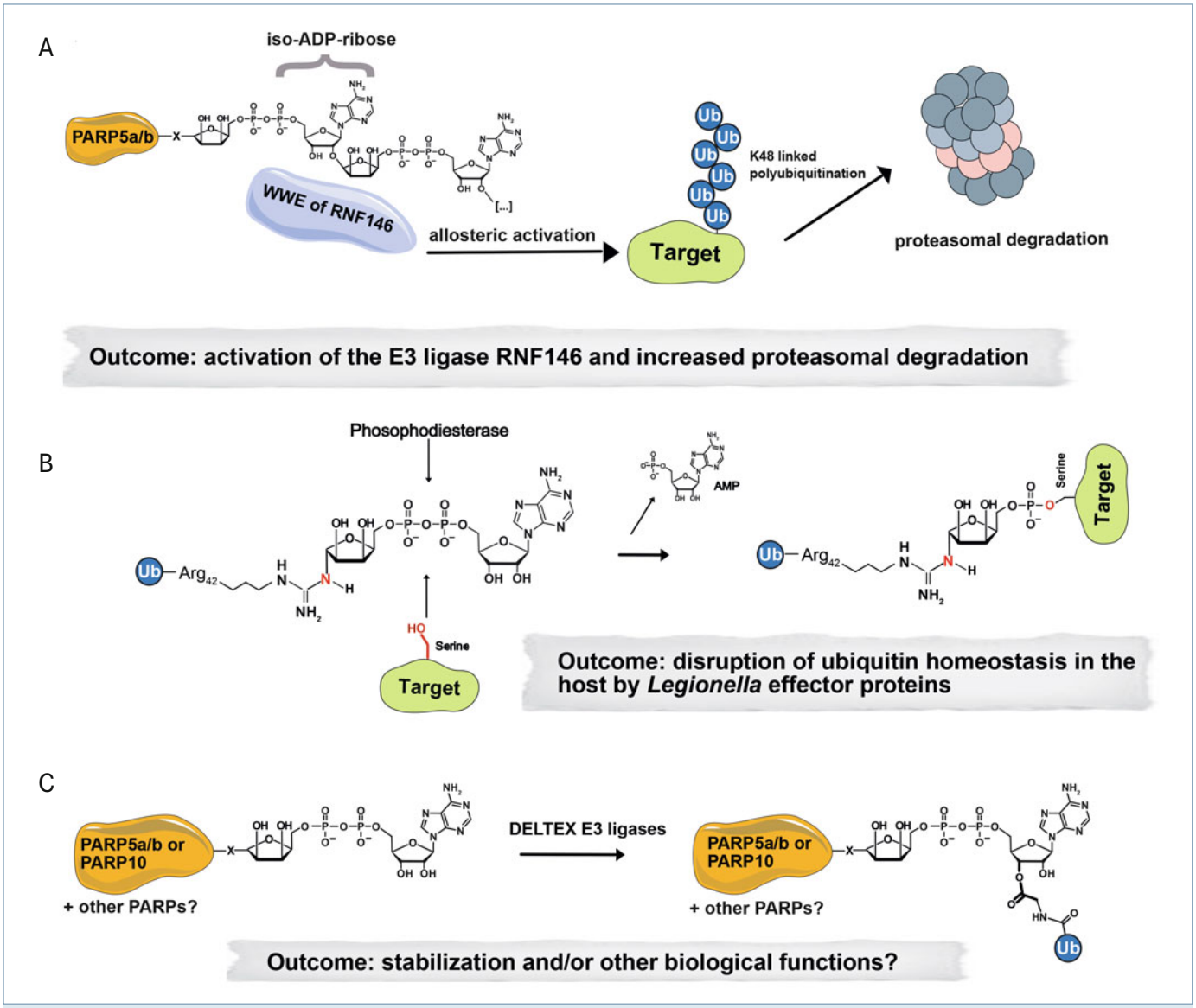
The 17 human PARP enzymes display striking differences in their subcellular localization (**Fig. 2**). Many PARPs typically localize in the nucleus or cytosol, and some shuttle between these compartments. The most active, abundant, and well-studied PARP enzyme, PARP1, is predominantly localized in the nucleoplasm and nucleolus, where it plays a critical role in processes such as DNA repair and transcriptional regulation. Similarly, PARP2 and PARP3 are primarily nuclear enzymes. PARP11, on the other hand has a unique and curious localization: it is situated at the nuclear periphery, where it might stabilize and remodel the nuclear envelope, possibly by MARylating nuclear pore complex proteins. Other PARPs, such as PARP7, PARP9, PARP10, and PARP14, can translocate to the nucleus under specific conditions, underscoring the dynamic nature of their localization [4].

In the cytoplasm, several PARPs are associated with cytoplasmic condensates of proteins and RNA that form in response to cellular stress – the so-called stress granules. This group includes PARP5a, PARP12, PARP13, PARP14, and PARP15, which contribute to stress granule assembly. PARP12, for instance, transitions from the Golgi complex to stress granules in a reversible manner and PARP13 plays a role in stress granule dynamics, further emphasizing the importance of PARPs in cytoplasmic stress responses.

PARP16 is the only known membrane-associated PARP in humans. It localizes to the endoplasmic reticulum (ER) membrane via a transmembrane helix, with its ART domain facing the cytoplasm. Additionally, PARP4 associates with vault particles, which are ribonucleoprotein complexes found in the cytoplasm, while the PARP-like protein NEURL4 has recently been linked to mitochondrial functions [4].

This complex and varied localization of PARPs suggests functional compartmentalization within the cell. However, overlapping spatial niches also point to potential functional crosstalk, particularly in cases where multiple PARPs coexist in the same subcellular environments.

Intriguingly, when the chemical versatility of ADPr meets the diverse range of localization of PARP enzymes, it enables spatiotemporal regulation of key biological processes,



▲ **Fig. 3:** Emerging crosstalk between ADPr and ubiquitination. **A**, E3 ubiquitin ligases with WWE domains, such as RNF146, recognize the iso-ADP-ribose in PAR. This interaction allosterically stimulates RNF146's polyubiquitination activity, promoting the degradation of its substrates. **B**, *Legionella pneumophila* disrupts the host's ubiquitin signaling pathways by ADP-ribosyling ubiquitin on arginine 42 (Arg42). Subsequently, its phosphodiesterase domain cleaves ADP-ribose at the pyrophosphate bond, and the resulting phosphoribose remnant is attached to a serine residue on the host target, generating a noncanonical ubiquitin-substrate linkage. **C**, E3 ubiquitin ligases from the DELTEX family add ubiquitin to the 3′ hydroxyl group of ADP-ribose linked to target proteins such as PARP5a, PARP5b and PARP10, resulting in the formation of ADP-ribosyl-ubiquitin hybrid modification.

such as DNA repair, transcription, cell division, protein degradation, innate immunity, and regulation of signaling pathways and stress responses, to name a few.

The emerging crosstalk with ubiquitination

Recent discoveries introduced an additional layer of complexity to ADPr signaling: its interplay with ubiquitination. Notably, certain E3 ubiquitin ligases, such as RNF146, possess WWE domains that recognize iso-ADP-ribose (found in PAR). This interaction allosterically stimulates RNF146 ubiquitination activity, leading to increased degradation of its substrates and linking ADPr-modified proteins to the ubiquitin-proteasome system (**Fig. 3A**, [13]).

ADPr can also interact covalently with ubiquitin. For example, *Legionella pneumophila* uses ADPr to trigger noncanonical ubiquitination of host proteins. During this process, ubiquitin is initially ADP-ribosylated at a specific arginine residue. This is followed by the cleavage of ADP-ribose at the pyrophosphate bond, allowing the resulting phosphoribose remnant to attach to a serine residue on a host substrate, forming a noncanonical ubiquitin-substrate linkage and thereby disrupting the host's ubiquitin signaling pathways **(Fig. 3B**, [14]).

Even more remarkably, recent research showed that ADP-ribose itself can serve as a substrate for ubiquitination, leading to a unique hybrid modification termed ADP-ribosyl-ubiquitin. In this modification, ubiquitin is attached directly to the ribose moiety of ADP-ribose, generating a hybrid structure. Specific E3 ubiquitin ligases from the DELTEX family have been identified as capable of adding ubiquitin to the 3′ hydroxyl group of ADP-ribose linked to target proteins,

resulting in the formation of ADP-ribosyl-ubiquitin chains *in vitro* (**Fig. 3C**, [15]). Last year, indirect evidence emerged regarding the involvement of PARP5a, PARP5b and PARP10 as acceptors of the hybrid ADP-ribose-ubiquitin modification within a cellular context [16]. While the specific functions of hybrid ADP-ribose-ubiquitin signals remain to be fully elucidated, one possibility is their role in recruiting specialized E3 ubiquitin ligases, such as RNF114 and RNF166, which possess tandem ADPr and ubiquitin recognizing domains [17].

Looking ahead

As research advancements simplify the exploration of mono-ADPr, mono-PARP enzymes are gaining attention, presenting exciting opportunities for new therapeutic approaches and enhancing our understanding of ADPr as a whole.

One of the foremost challenges in this field is to systematically elucidate the specific mechanisms that link individual modification events on distinct substrates to defined biological outcomes. Our lab is dedicated to this mission, aiming to unravel the hidden roles of mono-ADPr, particularly focusing on the functions of mono-PARPs across different organelles. We are specifically interested in mapping the precise localization of PARPs and mono-ADPr within organelles using advanced techniques such as STED microscopy. A particular area of interest is the intriguing localization of the PARP11 enzyme at the nuclear pore complex. By investigating this localization, we hope to gain novel insights into the function of PARP11.

Moreover, we are inspired by the growing evidence of hybrid ADP-ribosyl-ubiquitination. Our objective is to expand the understanding of this fascinating interplay of PTMs by identifying new substrates and illuminating how these modifications influence cellular processes. We believe that our efforts in this area will enhance our understanding of the broader biological implications of ADPr and ubiquitination in cellular regulation.

Ultimately, the beauty of ADP-ribosylation lies in its intricate complexity, which continuously inspires and challenges us.

Acknowledgements

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