[Frontiers in Bioscience 19, 1041-1056, June 1, 2014]

Identification of ADP-ribosylated peptides and ADP-ribose acceptor sites

Florian Rosenthal[1,2], Michael O. Hottiger[1]

[1]Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, Switzerland, [2]Life Science Zurich Graduate School, Molecular Life Science Program, University of Zurich, Switzerland

TABLE OF CONTENTS

1. Abstract
2. ADP-ribosylation
2.1 Bacterial ADP-ribosyltransferases
2.2. Mammalian ADP-ribosyltransferases
3. Readers of ADP-ribosylation
3.1. PAR-binding motifs (PBMs)
3.2. Macrodomains
3.3. The WWE domain
3.4. The PAR-binding zinc finger (PBZ)
4. ADP-ribose acceptor sites
5. Removal of ADP-ribosylation
5.1. Degraders of poly-ADP-ribosylation
5.1.1. Poly-ADP-ribose glycohydrolase (PARG)
5.1.2. ADP-ribosyl hydrolase 3 (ARH3)
5.1.3. Nudix hydrolases
5.2 Erasers of mono-ADP-ribosylation
5.2.1. ADP-ribosyl hydrolase 1 (ARH1)
5.2.2. Macrodomain proteins
6. Towards identifying the ADP-ribosylome
6.1. Identification of ADP-ribosylated proteins by protein microarrays
6.2. Enrichment strategies for ADP-ribosylated proteins
6.3. Mass spectrometry techniques for ADP-ribosylation research
6.4. Detection of ADP-ribosylated peptides from in vitro modified proteins by mass spectrometry
6.5. Identification of the ADP-ribosylome by mass spectrometry
7. Perspective
7.1. Identification of a conserved ADP-ribosylation motif
7.2. Functional contribution of the ARTD family members
8. Acknowledgement
9. References

1. ABSTRACT

ADP-ribosylation is a post-translational modification of proteins that comprises the transfer of the ADP-ribose moiety from NAD^+ to specific amino acid residues on substrate proteins or to ADP-ribose itself. It is catalyzed by ADP-ribosyltransferases, a family of currently 22 human proteins that all possess an ADP-ribosyltransferase catalytic domain. ADP-ribosylation is a reversible modification that can be hydrolyzed by ADP-ribosyl hydrolases. In order to define the functional role of cellular ADP-ribosylation and the functional contribution of distinct ARTD family members, it is necessary to identify all ADP-ribosylated proteins, as well as their modified residues in the context of different cellular conditions and stresses. Here, we summarize the most recent progress in defining the cellular ADP-ribosylome and the efforts to detect ADP-ribose acceptor sites by enzymatic reactions and mass-spectrometry.

2. ADP-RIBOSYLATION

Until now, proteomic studies have identified more than 200 different post-translational modifications (PTMs), which add a high level of complexity to the proteome, generating over 1 million different proteins from approximately 20,000 protein-coding genes (1, 2). PTMs allow the cell to adapt and cope with stress conditions such as environmental stimuli, pathogens or diseases. The best-studied PTMs are the covalent modification of amino acids with small chemical groups such as phosphate, acetate or methyl (3). ADP-ribosylation is an ancient PTM that is conserved in all organisms from bacteria to humans, except in yeasts, and is catalyzed by ADP-ribosyltransferases (ARTs) (4-6). ADP-ribosylation can be divided into two subtypes (7): mono-ADP-ribosylation, which defines a reaction where only one ADP-ribose group is transferred to the target protein amino acid (8), and poly-ADP-ribosylation (PARylation), which involves the transfer and

elongation of the initial ADP-ribose moiety to generate ADP-ribose chains. In both reactions, the acceptor site is initially mono-ADP-ribosylated, followed by an elongation reaction in the case of poly-ADP-ribosylation. In addition, the initially linear poly-ADP-ribose (PAR) can be branched, for which ADP-ribose chains are attached to the existing polymers in a non-linear manner (9). In contrast to highly transient PARylation, mono-ADP-ribosylation is a more stable modification. Therefore, the removal of the last, protein-bound ADP-ribose moiety is proposed to be the rate-limiting step in PAR hydrolysis (10). However, the recent discovery of specific mono-ADP-ribosyl hydrolases (see section 5.2.2) has moved the research on these enzymes forward and revealed important cellular functions of the mono-ADP-ribosylation cycle (11).

In addition to its role in the cellular stress response (12, 13), ADP-ribosylation regulates important cellular and biological processes such as replication, transcription, chromatin architecture, telomere maintenance, cell death, immune responses, and proteasomal functions (4, 14-22). At the molecular level, ADP-ribosylation affects the function of the modified proteins as well as complex formation with other factors that are thus directly or indirectly influenced by mono- or poly-ADP-ribosylation (23). ADP-ribosylation has been described to inhibit protein-protein or protein-nucleic acid interactions, leading for example to chromatin de-condensation or the release of DNA binding proteins from chromatin (24, 25). ADP-ribose also serves as a scaffold for the recruitment of proteins during complex formation, which is a well documented function mediated by ADP-ribose binding motifs (see section 3).

2.1. Bacterial ADP-ribosyltransferases

During host invasion, bacterial pathogens neutralize the phagocytic functions of immune cells, disrupt the cytoskeleton or inhibit signal transduction by secreting protein toxins that are able to mono-ADP-ribosylate intracellular host target proteins (26, 27). These toxin-mediated PTMs lead to the inactivation of the respective target protein, which disrupts cellular functions and causes diseases such as diphtheria and cholera (27). Bacterially encoded ARTs are well-studied and the acceptor amino acids for ADP-ribosylation have been defined in many cases. Based on structural aspects, bacterial toxins are divided into two main subclasses that harbor either an H-Y-E (found in the Diphtheria toxins) or an R-S-E motif (found in the Cholera toxin) in their catalytic domain (6).

2.2. Mammalian ADP-ribosyltransferases

Shortly after the identification of ADP-ribose as a PTM, the first enzyme capable of synthesizing PAR was discovered and named poly-ADP-ribose polymerase (PARP) (28). For nearly two decades, PARP was believed to be the only ADP-ribosyltransferase capable of synthesizing PAR. In the late nineties, with the sequencing of the human genome, additional genes that share the same catalytic domain were identified, suggesting that several other proteins might be able to synthesize PAR (29). Thanks to many global studies providing structure and sequence information about the proteome, 22 human proteins that harbor a catalytic domain similar to the one found in PARP have been identified up to now (6). Based on available amino acid sequences and crystal structures, the mammalian ARTs can be grouped into two groups that differ in their catalytic motif (6). One group shares structural similarities with Cholera toxin and is therefore referred to as ADP-ribosyltransferase Cholera toxin-like (ARTC). Currently, human ARTCs comprise of five members (ARTC1-5), which are all located at the cell membrane facing the extracellular space (6). The second group shares structural similarities to Diphtheria toxin and is therefore referred to as ADP-ribosyltransferase Diphtheria toxin-like (ARTD). Eighteen different human ARTD members (ARTD1-18, ARTD1 representing the initially identified PARP protein) are currently known and found in different cellular compartments. The ARTD family can further be subdivided based on differences in the amino acid sequence of the catalytic core domain. ARTDs that were proposed to synthesize PAR are characterized by the presence of the conserved amino acid triad H-Y-E, whereas mono-ADP-ribosyltransferases lack the glutamate residue in position 3 of the triad (6). The members of the ARTD family are sub-divided into three different groups based on their biochemical properties: the family-founding enzyme ARTD1 (PARP1), as well as ARTD2 (PARP2), generate branched PAR and are representative of the first group. ARTD1 is the most abundant nuclear ARTD family member and accounts for approximately 90% of the PAR formation in a cell (30), whereas ARTD2 is less abundant, less active and responsible for the majority of the remaining PAR formation in cells that lack ARTD1 (30, 31). Although ARTD3 harbors the H-Y-E triad, its enzymatic property is still under debate. Both mono- and poly-ADP-ribosylation activity have been reported for ARTD3 (32, 33), suggesting that beside the H-Y-E triad, other amino acids or specific conditions are most likely also important for PAR formation. A second group of enzymes consists of the telomere-associated ARTD5 and ARTD6 (Tankyrase1 and 2), which like ARTD1, 2 and 3 are also able to modify themselves as well as several target proteins (34, 35). In contrast to the first group, ARTD5 and ARTD6 are oligo-ADP-ribosyltransferases, which synthesize linear ADP-ribose chains with a maximum length of 20 units (35). The third group contains enzymes that lack the glutamate residue in the above-mentioned triad motif, limiting them to be mono-ADP-ribosyltransferases, as shown for ARTD8 (PARP14) and ARTD10 (PARP10) (36). Moreover, although all identified ARTD family members share a common catalytic domain, it is not clear whether all proteins are enzymatically active (6). Recent studies provided evidence that ARTD9 and ARTD13 do not posses catalytic activity (36, 37). For the other mono-ADP-ribosyltransferases of the ARTD family, a catalytic activity was postulated, but still lacks experimental validation (6). Recent overexpression and knockdown studies of all intracellular ARTD family members in HeLa cells revealed that most ARTDs are localized in the cytoplasm, that the ratio of nuclear to cytoplasmic poly(ADP-ribose) varies throughout the cell cycle and that four phenotypic classes of ARTD functions exist (38). These functions are related to the regulation of membrane structures, cell viability, cell division and influence the actin cytoskeleton.

3. READERS OF ADP-RIBOSYLATION

PTMs modulate the activity of target proteins either directly or indirectly by affecting complex formation with other proteins. An important concept in the realm of PTMs is thus the recognition and specific binding of a given modification by 'reader' proteins. In the case of ADP-ribosylation, a large number of PAR binding proteins is recruited to modification sites where they function in a modification-dependent manner (16, 39). Therefore, non-covalent binding of reader proteins to ADP-ribosylated targets is an important mechanism for mediating many of the regulatory functions of ADP-ribosylation (40). PAR-binding domains that have been described so far are the PAR-binding motifs (PBMs), macrodomains, PAR-binding zinc-fingers (PBZs) and the WWE domains (23, 39, 41).

3.1. PAR-binding motifs (PBMs)

The first motif described to interact with PAR was the PBM motif found in various proteins of the DNA damage signaling network and in DNA repair factors (e.g., p53, XRCC1, MRE11, ATM, Ku70/86 and DNA-PK) (29, 42, 43). The PBM consists of a stretch of amino acids with the sequence $[HKR]_1$-X_2-X_3-$[AIQVY]_4$-$[KR]_5$-$[KR]_6$-$[AILV]_7$-$[FILPV]_8$ (23, 43). The PBM non-covalently interacts with free PAR *in vitro,* which is postulated to act as a scaffold for the recruitment of different DNA-repair proteins or function as a signaling factor that is sensed by PBM domain-containing proteins. This mechanism has so far only been described in a purely biochemical system and needs to be further validated *in vivo.*

3.2. Macrodomains

The macrodomain consists of 130-160 amino acids and displays a mixed a/ß-fold that is conserved in over 300 currently known macrodomain-containing proteins from viruses and bacteria to humans (23, 44). The first human macrodomain was identified in the histone variant macroH2A (45). Until now, ten genes encoding 14 different macrodomain-containing proteins, harboring one to three folds, have been predicted by sequence homology in humans (46, 47). A subgroup comprised of human ARTD8, MacroD2, and viral SARS-CoV has been shown to efficiently bind to mono-ADP-ribosylated proteins (48-51). In contrast, PAR-binding has been observed for human macroH2A1.1 and the macrodomain of ARTD9, as well as for the viral SFV protein. A third group, able to bind both mono-ADP-ribose and PAR, contains human MacroD1, viral HEV, as well as archaeal Af1521 (49, 50, 52). Since it has been suggested that macrodomains bind to the terminal ADP-ribose of PAR (last moiety of the chain), it is likely that the differences in the binding of mono- and/or poly-ADP-ribose are due to steric reasons.

3.3. The WWE domain

The WWE domain is a globular domain that is defined by two conserved tryptophans (W) and a glutamic acid (E) residue and is usually found as a single or double domain. So far, WWE domains have been identified in two protein families; the E3 ubiquitin ligases (e.g., RNF146, DELTEX1 or Trip12) and the ARTDs (ARTD8 and ARTD11-14) (6, 23, 53). The fact that all WWE domain-containing proteins interact with ADP-ribose and either have ubiquitination activity or are members of the ARTD family, suggests that both mechanisms are tightly linked with and influence each other. For example, ADP-ribosylation could be a signal or recruiting factor for E3 ubiquitin ligases, which subsequently mark proteins for degradation or influence their properties (54). WWE domains have the ability to interact with iso-ADP-ribose within the PAR chain (53, 54). Some WWE domains have mutations in their binding motif that abolish PAR binding, but might turn them into mono-ADP-ribose binders (54, 55). Examples are the WWE domains of DDHD2 or ARTD8 and, depending on the study, also of ARTD11, which are believed to bind the terminal ADP-ribose as well as a mono-ADP-ribose modification.

3.4. The PAR-binding zinc finger (PBZ)

The PAR-binding zinc finger (PBZ) is a small unordered fold with a zinc-coordinated backbone that can interact with one or two ADP-ribose units in a PAR chain and the connecting pyrophosphate bond as well as with the a(1->2) O-glycosidic bond between the ADP-ribose moieties (23, 56, 57). So far, PBZ domains have only been identified in a few DNA-damage response proteins, like aprataxin and PNK–like factor (APLF), or in checkpoint with forkhead-associated and ring finger domains (CHFR) (56, 57). Both proteins are recruited to sites of DNA damage in a PBZ- and PAR-dependent manner. Misregulation of CHFR is implicated in tumor progression, most probably due to its function at the antephase checkpoint, which is malfunctioning upon loss of the PBZ domain (56, 58).

4. ADP-RIBOSE ACCEPTOR SITES

In the case of bacterial ARTs, the acceptor amino acids have been successfully mapped because most of these toxins modify only a single protein or a class of proteins at a single specific residue (26, 27). Most sites have been identified indirectly via chemical protection of potential acceptors, Edman-degradation, thin layer chromatography or other chemical methods and have later been confirmed by site directed mutagenesis (59-61). This led to the identification of several sites with the main acceptors being arginine, asparagine, dphtamide and cysteine (26). More recently, a study using mass spectrometry has identified threonine 148 of actin and glutamine 61 and 63 of RhoA as ADP-ribose acceptors (62).

Despite substantial efforts, the identification of the amino acids in mammalian proteins specifically modified by a distinct ARTD has not been possible so far. Over thirty years ago, mammalian ADP-ribose acceptor sites were primarily characterized based on the chemical properties and stability of the modification on ARTD1, core histones and linker histone H1 (63-65). As the majority (over 70%) of the analyzed ADP-ribose modifications were sensitive to alkali and neutral NH_2OH, but stable to acid, a carboxyester linkage was suggested (65). A linkage with such chemical properties points to glutamate or aspartate residues as acceptors, which has been believed for many years without further validation.

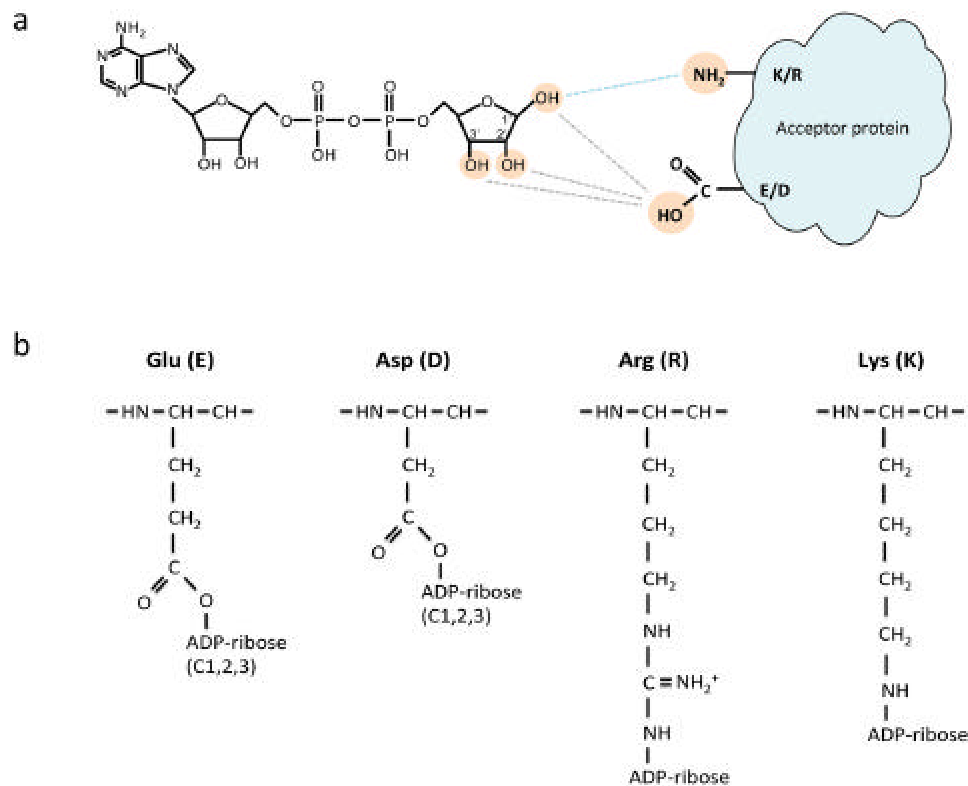

Figure 1. ADP-ribose linkage types depend on the acceptor site. a) The protein-bound ADP-ribose moiety can be linked to the acceptor site via the C1, C2 or C3 atom. The linkage to arginine (R) residues was identified as C1, which may also be the case for lysines (K). However, for glutamate (E) and aspartate (D), no final evidence has been provided so far. b) The best-characterized ADP-ribose acceptors are the acidic residues aspartate and glutamate, as well as the basic residues arginine and lysine. While in the first case a carboxylate ester linkage is formed, the latter two residues lead to N-glycosidically-linked ADP-ribose.

The remaining 30% of the modifications were NH_2OH-resistant and 15% were even alkaline resistant, clearly indicating that other linkages must exist (64). Later, experiments with two ARTCs from avian erythrocytes suggested that the NH_2OH-resistant and alkali-stable fraction represents an ADP-ribose-arginine linkage (65-67). However, under certain conditions NH_2OH treatment was also reported to release ADP-ribose from modified arginine residues (68). Together, these early observations provide clear evidence that chemical stability is not a reliable criterion to distinguish between different ADP-ribose acceptors sites, calling for other approaches to discriminate between different acceptor sites. More recent work using site-directed mutagenesis studies of ARTD1 and ARTD2 have identified lysine residues as ADP-ribose acceptors (69). In contrast, site-directed mutagenesis experiments with ARTD10 have identified glutamates as acceptors sites (36). As these studies have relied on mutagenesis experiments that can potentially alter the protein structure and therefore introduce a bias, methods based on mass spectrometry are needed to unequivocally identify ADP-ribose acceptor sites (see 6.3).

Moreover, the linkages within PAR or between ADP-ribose and the acceptor residue can potentially be of different chemical nature and therefore exhibit different biochemical properties. The ribose 5-phosphate of ADP-ribose has three potential linkage acceptors, which are the OH-groups at the C1, C2 and C3 position (63) (Figure 1a). Preliminary studies suggested an attachment at the C1 position in the case of modified glutamates, but these results only provided indicative, but not conclusive evidence (63) (Figure 1b). In contrast, mono-ADP-ribosylation of arginines has been analyzed by NMR and could be identified as being C1 linked (70) (Figure 1b). Furthermore, it was shown that the enzymatic reaction results in a ribosyl-guanidinium linkage in a conformation, which undergoes anomerization to a ß linkage in a 1:1 ratio under physiological conditions (70). All these results illustrate the high complexity of ADP-ribosylation (Figure 1). Furthermore, while research has focused on enzymatically-catalyzed ADP-ribosylation, non-enzymatically-mediated, yet still covalent ADP-ribosylation has also been described (71, 72). ADP-ribose is a potent histone glycation and glycoxidation agent *in vitro* (71). Glycation is the covalent binding of an ADP-ribose molecule through Schiff base formation to lysine residues, which is stable upon treatment with hydroxylamine. Incubation of ADP-ribose with histones H1, H2A, H2B, and H4 *in vitro* at pH 7.5 and 37°C over

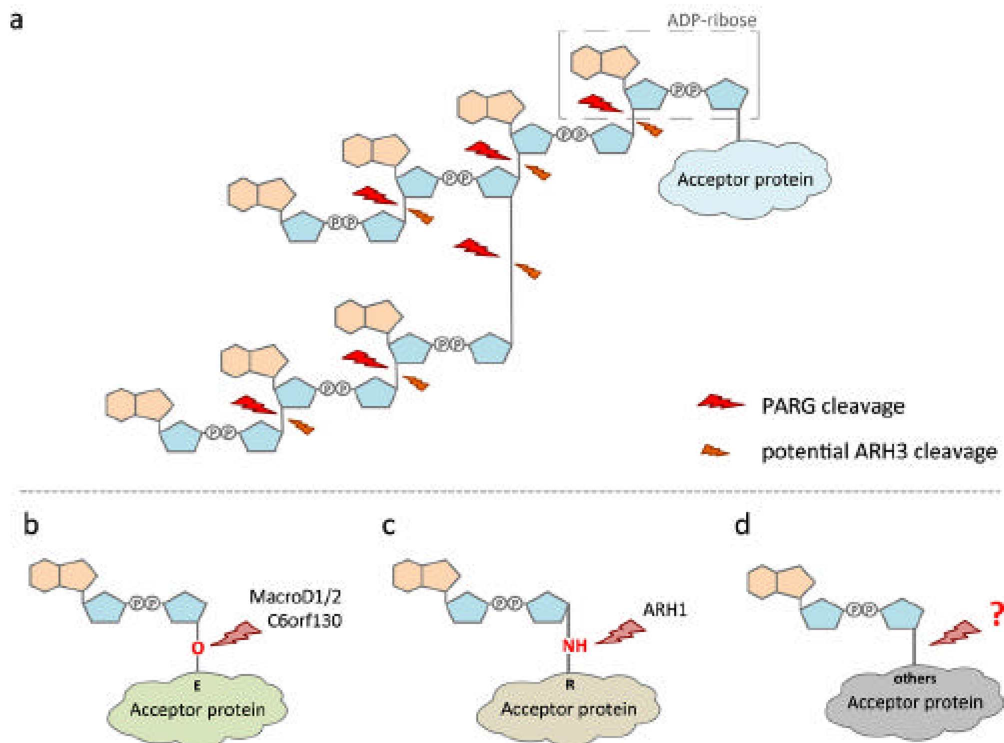

Figure 2. Degradation and erasing of ADP-ribose modifications. Schematic representation of mono- and poly-ADP-ribose and the points of attack for known catabolic enzymes. a) The ADP-ribose units within PAR are cross-linked via the C1 atom, while branch points are created at the C2 atom. For simplicity, the protein linkage is only depicted as being C2 linked, nevertheless, other linkages might also occur. b) The mono-ADP-ribosylated acidic residue glutamate (E) is recognized and erased by macrodomain proteins. For simplicity the protein linkage is only depicted as being C2 linked, nevertheless, other linkages might also occur. c) The mono-ADP-ribosylated basic residue arginine (R) is linked via C1 and recognized and erased by ARH1. d) The erasers of other known acceptors for ADP-ribose, such as lysine (K) and aspartate (D), remain elusive so far.

night resulted in the formation of ketoamines by Amadori rearrangement of the formed Schiff base (71, 73, 74). In the case of an Amadori rearrangement the attached ADP-ribose would be linked via a C1 linkage (74). Based on the long incubation time, which is substantially longer than common incubation times of ADP-ribosylation reactions, glycation only needs to be considered in biological systems, if very high concentrations of free ADP-ribose are generated from NAD^+ or PAR. The specific types of linkages have potentially far-reaching biological implications, since they may affect the removal of different modifications by ADP-ribosyl hydrolases.

5. REMOVAL OF ADP-RIBOSYLATION

ADP-ribosylation regulates many important biological processes and serves as a specific signal. However, for the functioning of a cell, it is not only important that the signal is synthesized (written) in a coordinated and specific manner, but it must also be recognized by readers (see section 4) and be removed (erased) in a timely manner. Indeed, synthesis and degradation of PAR is a highly dynamic process with a half-life of only a few minutes (75). Identifying and studying the enzymes that degrade PAR and erase the ADP-ribose modifications is thus of central importance for our understanding of the dynamics and the cellular functions of ADP-ribosylation.

5.1. Degraders of poly-ADP-ribosylation

The enzymatic activities that degrade PAR to ADP-ribose are currently poorly understood. This is partly due to the difficulty to analyze the different ADP-ribose structures, especially the complex linear- and branched poly-ADP-ribose (Figure 2a).

5.1.1. Poly-ADP-ribose glycohydrolase (PARG)

PARG is the best-studied PAR-degrading enzyme so far and is composed of at least five isoforms: A nuclear 110-111 kDa isoform, three cytoplasmic isoforms and a mitochondrial isoform (76, 77). All of these isoforms result from alternative splicing or from translation re-initiation of a single gene locus (14, 78). PARG is the enzyme responsible for the majority of the PAR-degrading activity in cells. It hydrolyses the 2'–1'' glycosidic ribose-ribose bonds of PAR into free ADP-ribose moieties (Figure 2a), which can function as second messenger in various compartments of the cell or can be recycled into ATP (14, 78). Recent structural studies have elucidated the enzymatic reaction mechanism and have identified several moieties that are important for PAR-binding and hydrolysis (79, 80). The terminal ribose of the PAR chain is bound by PARG

and the ribose-ribose O-glycosidic linkage forms an oxocarbenium intermediate with E115 in the catalytic core. Subsequently, a water molecule attacks the intermediate, which results in the release of an ADP-ß-ribose'' (79). Some studies have proposed that PARG exhibits an endo-activity besides the exo-glycohydrolase activity that is weak, however (81, 82). In contrast, earlier product analysis experiments revealed that protein-bound PAR is mainly cleaved endoglycosidically into large fragments, which can subsequently be further processed by endo- and exo-glycosidic digestion into single ADP-riboses (83). Recently, the catalytic center of PARG has been reported to have structural homology to macrodomains (79). Due to steric hindrance, the protein-bound ADP-ribose was proposed to not be hydrolyzed by PARG, resulting in a mono-ADP-ribosylated protein (81).

5.1.2. ADP-ribosyl hydrolase 3 (ARH3)

ARH3, another enzyme structurally distinct from the PARG proteins, was also found to only degrade PAR (but not to reverse mono-ADP-ribosylation) and in a manner similar to PARG, although most PAR-hydrolyzing activity of the cell is assigned to PARG (84, 85) (Figure 2a). As there is no structural similarity between the catalytic domains of ARH3 and PARG, (85, 86), the presence of two independent and different mechanisms of PAR degradation in human cells is likely and possibly also functionally relevant.

5.1.3. Nudix hydrolases

In humans, an additional family of enzymes capable of hydrolyzing ADP-ribose exists. In contrast to the above-mentioned enzymes, the nudix box-containing enzymes do not remove protein bound ADP-ribose or PAR, but hydrolyze the pyrophosphate bond within ADP-ribose, generating AMP and ribose 5-phosphate (87). The nudix superfamily is conserved in all kingdoms of life and is defined by its characteristic 23 amino acid nudix box motif $Gx_5Ex_5[UA]xREx_2EExGU$, where U is a hydrophobic amino acid (87, 88). Nudix proteins are pyrophosphohydrolases, which act on a variety of substrates containing a nucleoside diphosphate linked to another moiety (87). In humans, NUDT5 and NUDT9 were shown to act as ADP-ribose pyrophosphatases, but NUDT5 has other substrates in addition to ADP-ribose (89-91). Both enzymes were suggested to protect proteins from non-enzymatic glycation by free ADP-ribose, by controlling the free ADP-ribose level in the cell. On the other hand, nudix proteins have also been shown to be inhibited by cytotoxic compounds (e.g., H_2O_2), a process that leads to accumulation of free ADP-ribose in addition to the enhanced PAR formation under stress (91).

5.2. Erasers of mono-ADP-ribosylation

The bonds within the PAR chain are of a different chemical nature than the first linkage between the acceptor site on the modified protein and the ADP-ribose. Consequently, most, if not all enzymes that degrade PAR leave the protein-bound ADP-ribose unit unchanged. The removal of this last ADP-ribose from the acceptor amino acid is the final and rate-limiting step of the de-ADP-ribosylation reaction. The last decade of ADP-ribosylation research has made it clear that different amino acids can serve as ADP-ribose acceptors. The differences in the chemical nature of acceptor residues such as lysines or glutamates, for example, imply that the chemical linkage between the acceptor side chain and the first ADP-ribose moiety must be chemically different. For example, an acidic amino acid would result in an ester bond, while at basic amino acids the ADP-ribose is linked via a N-glycosidic linkage (Figure 2b). Given these differences, one would anticipate that different classes of erasers exist that remove ADP-ribose modifications from distinct acceptor sites.

5.2.1. ADP-ribosyl hydrolase 1 (ARH1)

In the early nineties, a new hydrolase for mono-ADP-ribosylated arginine residues was purified and characterized (92). It was identified as an ADP-ribosylarginine-specific hydrolase *in vitro* and represented the founding member of the family of ADP-ribosylhydrolases (ARHs). Later, two more members of this family were identified by *in silico* sequence analysis and accordingly named ARH2 and ARH3 (86). Further studies revealed that ARH1 is indeed a mono-ADP-ribose hydrolase able to cleave ADP-ribose-arginine catalyzed by the bacterial cholera toxin (84) (Figure 2b). In contrast, no evidence for an enzymatic activity of ARH2 could be found so far (84). Until now, ARH1 is the only enzyme able to revert mono-ADP-ribose of a modified arginine residue, which may have derived from the action of ARTC family members (86, 93, 94). However, since ARH1 and the ARTCs are not localized in the same cellular compartment, an as yet unidentified arginine-specific intracellular ART might synthesize the ARH1 substrate. Very little is known about the biological function of the ARH protein family *in vivo,* but loss of ARH1 renders mice more prone to tumor formation (95), indicating that the balance between ADP-ribose synthesis and degradation is a critical mechanism for guaranteeing proper cellular functions and survival.

5.2.2. Macrodomain proteins

Interestingly, some macrodomain proteins also exhibit a catalytic function in addition to their ability to bind to ADP-ribose. Human MacroD1, MacroD2, orphan macrodomain C6orf130, as well as *E.coli* YmdB, are able to deacylate O-acetyl-ADP-ribose (OAADPr) (96, 97). OAADPr is the product of a protein deacetylation reaction, catalyzed by the NAD^+-dependent sirtuins (98). Macrodomains deacylate OAADPr, thereby producing ADP-ribose and acetate (97). Additional properties, which are poorly understood and investigated, include binding of OAADPr and poly(A) as well as ADPR-1''P phosphatase activity (46, 50). Recently a new enzymatic mechanism of MacroD1, MacroD2, C6orf130 and Af1521 has been investigated, identifying these proteins as novel glutamate-specific mono-ADP-ribosyl hydrolases (68, 99) (Figure 2b). It could be shown that macrodomain-containing proteins remove mono-ADP-ribose from *in vitro*-modified ARTD10 and histone proteins, as well as from PARG-treated ARTD1, although to a lesser extent. These different susceptibilities to macrodomain-mediated hydrolysis imply that ARTD1 and ARTD10 modify, at least in part, different residues. One study has proposed a C1 linkage of the ADP-

ribose to the glutamic acid acceptor residue (99), whereas a second model points to C2 (68), but the final proof for either of these possibilities has not been made. To finally answer the question, which C-atom of the distal ribose is attached to the glutamic acid, subsequently hydrolyzed by macrodomains, requires further investigations. However, with these findings, a major gap in understanding glutamic acid-modified ADP-ribose metabolism could be closed. The identification of new ADP-ribosyl hydrolases not only completes the ADP-ribosylation cycle, but also reveals important, biologically and medically relevant regulatory functions of cellular ADP-ribosylation (100). Along this line, MacroD2 could revert the inhibitory ADP-ribosylation of the disease-relevant kinase GSK3ß and thereby restore its activity *in vitro* and in cells (68). Furthermore, a deficiency in C6orf130 leads to neurodegenerative diseases (101). The observed defects in patients could be linked to enzymatic mutants of C6orf130 and thus also underline the importance of mono-ADP-ribosyl hydrolysis in humans. Based on the ability of C6orf130 to remove the intact PAR-chain from ARTD1, C6orf130 was named terminal ADP-ribose protein glycohydrolase (TARG1) (101). As this finding could not be reproduced yet, the existence of a TARG has to be further investigated (79) (and own observations). Moreover, it remains to be clarified, whether other acceptor residues, such as lysine, or asparagine, are irreversibly modified or whether other, yet unidentified enzymes erase these modifications (Figure 2b).

6. TOWARDS IDENTIFYING THE ADP-RIBOSYLOME

The tools to study ADP-ribosylation are currently very limited. Especially the lack of suitable antibodies for mono-ADP-ribosylation or for site-specific modifications restricts the analysis of ADP-ribosylation in complex biological systems. Until now, most studies that have identified ADP-ribosylated proteins and their ADP-ribose acceptor sites were performed in a targeted manner, analyzing only a few candidate proteins. It is therefore of great importance to establish new methods for the analysis of ADP-ribosylated proteins and for the identification of their modification acceptor sites. Advances in proteomics technologies, especially in mass spectrometry (MS), and the use of ADP-ribose readers as tools to purify or enrich ADP-ribosylated proteins, has recently opened exciting possibilities to study this complex protein modification. These systemic studies have broadened our horizon on the extent of the ADP-ribosylome and of ADP-ribosylation in the cell.

6.1. Identification of ADP-ribosylated proteins by protein microarrays

Protein microarrays are a powerful option to study protein interactions and modifications on a large scale and have been already successfully applied for the identification of kinase substrates (102). Recently, two studies have employed ProtoArrays®, covering around 8’000 proteins immobilized on glass slides to identify target proteins for ARTD2, ARTD8 and ARTD10 (103, 104). In both cases, recombinant ARTDs were used to modify the spotted proteins *in vitro* with biotinylated-NAD^+ and modified proteins were subsequently visualized by fluorescence-labeling of the biotin moiety in ADP-ribose. Of the 8’000 spotted proteins, 51 were ARTD2 substrates, 142 were ARTD8 substrates and 78 proteins were modified by ARTD10. Among the identified proteins were targets already known, confirming the applicability of protein microarrays for ADP-ribosylation target identification. As protein microarrays are only applicable for *in vitro* approaches, alternative techniques are key to obtain further insights into cellular ADP-ribosylation.

6.2. Enrichment strategies for ADP-ribosylated proteins

Since the fraction of cellular proteins with a particular modification is usually very small, researchers commonly use antibodies or other enrichment strategies specific for certain PTMs. This reduces the complexity of the cell lysate required for the subsequent identification of the proteins and their modification sites by mass spectrometry. A prominent example is the well-studied phospho-proteome (105, 106). Due to the availability of only one antibody against PAR and the lack of good antibodies against mono-ADP-ribosylated proteins, alternative methods for the identification of ADP-ribosylated proteins had to be established. Up to now, several studies attempted to identify the cellular ‘ADP-ribosylome’ by employing different methods.

In an elegant study the clickable NAD^+ analog 6-alkyne-NAD was used for the identification of ARTD1 substrate proteins (107). ARTD1 knockdown lysates were supplemented with ARTD1 and 6-alkyne-NAD, which subsequently was conjugated to Biotin-N3 in a click chemistry reaction. The modified proteins could then be purified with streptavidin and analyzed by mass spectrometry. This assay identified over 70 proteins, with a partial overlap to the study described earlier. However, since the NAD analogue is spiked into a lysate, which no longer contains cellular compartments and does not resemble the natural state of the cell, the results might be prone to the identification of false positive hits.

An important method for the enrichment of ADP-ribosylated proteins is the use of immobilized boronate derivatives (108, 109). Boronic acid groups form covalent esters with *cis*-diol groups that are present in various sugars and can therefore be used for the enrichment and monitoring of sugar-containing compounds (110, 111). Many decades ago, boronic acid-based enrichment led to the identification of the first ADP-ribosylated histones from cells (108, 109). Recently, the use of boronic acid was reintroduced into the ADP-ribosylation field by its successful application for the enrichment and mass spectrometric analysis of glycated proteins (112-114). In contrast to other enrichment strategies, boronic acid does not discriminate between mono- and poly-ADP-ribosylated proteins (108) and can therefore be applied to analyze the whole population of ADP-ribosylated proteins. Nevertheless, different drawbacks complicate the use of boronic acid enrichment. In particular, different derivatives and immobilizations can lead to varying specificities for different sugars and to differences in the pH-dependency of the binding reaction (115, 116).

Antibody-based enrichment protocols suffer from the lack of antibodies for mono-ADP-ribosylation. However, a study has employed the monoclonal PAR-binding antibody 10H (117) to enrich poly-ADP-ribosylated proteins from cellular extracts (43). To increase the amount of ADP-ribosylated proteins, PARG knockdown cells were treated with 100 µM N-methyl-N'-nitro-N'-nitroso-guanidine (MNNG) to induce genotoxic stress. Modified proteins were enriched by immunoprecipitation using the 10H antibody, under conditions still allowing indirect complex formations, and afterwards separated by SDS-PAGE. This procedure led to the identification of over 300 PAR-associated proteins with various cellular functions. The majority consisted of proteins involved in the DNA damage response, DNA and RNA metabolism and regulation, as well as cell death and cell cycle regulation (43). This study tremendously increased the number of identified PAR interacting proteins, but unfortunately could not fully distinguish between poly-ADP-ribosylated proteins and proteins that are forming a complex with modified proteins. Furthermore, mono-ADP-ribosylated proteins could not be identified due to the fact that the 10H antibody only binds to poly- and not oligo- and mono-ADP-ribose modifications (117).

An alternative method for the enrichment of ADP-ribosylated proteins is the use of the above-mentioned, ADP-ribose-binding macrodomain Af1521 (118, 119). In an initial study, modified proteins were enriched with Af1521, separated by SDS-PAGE, and identified by mass spectrometry (120). To reduce background, a non-binding mutant of Af1521 was used in a pre-clearing step. This method led to the identification of ADP-ribosylated proteins, including previously known targets such as ARTD1, Elongation factor-1-a and GDH1. However, the fact that the binding occurred under native conditions allowed also an indirect enrichment of proteins interacting with PARylated proteins. More recently, the same method was used in combination with stable isotope labeling by amino acids in cell culture (SILAC) and mass spectrometry (119).

6.3. Mass spectrometry techniques for ADP-ribosylation research

Today, the identification and characterization of PTMs is facilitated by mass spectrometry and a vast amount of information about various modifications and their functions is available because of these techniques (121, 122). Prominent examples are phosphorylation, ubiquitination and glycosylation, where great advances were made in the recent years (123-125). Therefore, also the field of ADP-ribosylation is in need for novel protocols and methods to enter this exciting development and to gain a broader insight into the dynamics of ADP-ribosylation.

Earlier studies on ADP-ribosylated substrates concluded that the site identification with state-of-the-art mass spectrometry techniques at that time was not possible (126). Furthermore, detailed studies on the fragmentation behavior of ADP-ribose and modified peptides revealed that the modification is almost completely fragmented under conventional MS conditions using collision-induced dissociation (CID) (127). Although the fragmentation profile always generated the same fragments, with ions indicative for ADP-ribosylated peptides, the fragmentation of the peptide backbone by CID was too weak to gain enough information of the peptide sequence and to allow for the determination of the modification site. Furthermore, in most cases ADP-ribose is completely fragmented by CID fragmentation and no rest remains on the amino acid. Therefore, identification of the acceptor site within an ADP-ribosylated peptide by CID-MS has remained challenging.

Recently, novel fragmentation techniques for the identification of ADP-ribosylated peptides by MS have been established. Electron capture dissociation (ECD) is a non-ergonic process, leading to a high proportion of random peptide backbone cleavages during fragmentation (128). This process is especially suited for highly charged peptides, since it leads to the stabilization of labile PTMs on the peptide and to a better *c* and *z* ion series, which allows better sequence assignments (129). When ECD was applied to a chemically ADP-ribosylated standard peptide containing only arginine as a potential acceptor, the modification as well as its site could be readily annotated, underlining the power of this method (127). A second fragmentation method that uses a comparable chemistry to ECD is electron transfer dissociation (ETD). The advantage of ETD is its better availability and accessibility due to lower machine costs (130). ETD has also been successfully applied to chemically ADP-ribosylated peptides and more complex protein mixtures and enabled the identification of modified arginine as well as lysine residues (131, 132). The first study that used ETD to detect ADP-ribosylation sites on histones enzymatically modified by ARTD1 *in vitro,* identified lysine residues as acceptors and thereby showed the robustness of the ETD approach (133).

A third fragmentation technique that was recently introduced is higher energy collisional dissociation (HCD) (119, 134). In comparison to CID, the advantage of HCD is a better coverage of the whole mass range and the ability to acquire MS and MS/MS spectra at high resolution, thereby recording smaller fragment ions, which leads to better sequence and PTM assignment (135, 136). HCD thus seems to be a promising fragmentation technique for ADP-ribosylation studies.

6.4.Detection of ADP-ribosylated peptides from *in vitro* modified proteins by mass spectrometry

As PAR is very complex due to its length and branched structure, the detection of PARylated peptides by MS/MS is very challenging. To overcome this drawback, the complexity of PAR can be reduced *in vitro* by different methods. The pre-treatment of the samples with PARG reduces the modification to one protein-bound ADP-ribose moiety (see above). An alternative option is the enzymatic reduction of PAR by cleaving the pyrophosphate bond of ADP-ribose by phosphodiesterase 1 (PDE1) *in vitro*. This reaction reduces the complexity of the modification and turns it into a chemically more stable group (i.e., phospho-ribose). The newly formed terminal phosphate group can be

used to enrich modified peptides with conventional phosphopeptide enrichment strategies. A recent study identified several glutamic acid as well as lysine residues in ARTD1 as potential acceptors; however, the unambiguous annotation of the exact modification site was not possible due to a lack of sequence information in the CID spectra (137). An additional approach reduced the complexity of PARylation by using a mutated ARTD1 carrying an E to Q mutation at position 988, which hinders PAR formation, making ARTD1 E988Q an inefficient mono-ADP-ribosyltransferase (138, 139). With this approach, D387, E488 and E491 within the automodification domain of the ARTD1 E988Q mutant could be identified as auto-ADP-ribosylation sites by CID mass spectrometry (140). However, it remains to be clarified, whether mutating important amino acids of the catalytic domain of ARTDs is not changing the specificity of the catalytic reaction.

6.5. Identification of the ADP-ribosylome by mass spectrometry

In a more global analysis, the above-introduced Af1521-based enrichment method was utilized to identify the ADP-ribosylome from cells that underwent different types of genotoxic stress (119). The use of H_2O_2, MMS, UV-irradiation and ionizing irradiation identified a total of 235 stress-induced ADP-ribosylated proteins, but not their ADP-ribose acceptor sites. The identified proteins are mainly localized in the nucleus and involve numerous previously identified targets. The identified target proteins could furthermore be functionally sorted into two main groups: the well established group of proteins involved in DNA metabolism, involving players of the DNA repair, DNA replication as well as chromosome organization and proteins involved in RNA metabolism features like transcription, processing and splicing of RNA. In contrast to DNA damage, RNA metabolism was so far not as extensively linked to ADP-ribosylation, therefore, these results open new and interesting possibilities in the field of ADP-ribosylation research.

A second global dataset of ADP-ribosylated peptides and their modification sites was generated with a novel combination of enrichment and chemical disruption of the ADP-ribose (141). ADP-ribosylated peptides were enriched by boronate affinity chromatography, in which boronic acid groups covalently bind 1,2-*cis*-diols that are present in sugars, allowing for the enrichment of ADP-ribosylated peptides (112, 142). As the ester bonds between the ADP-ribose and the acceptor amino acids glutamate and aspartate are labile in the presence of NH_2OH (143), peptides modified at these residues could be eluted by adding NH_2OH. Interestingly, not the whole modification is removed, but a 15 Da mass shift is detected, allowing the determination of the ADP-ribosylation site within the enriched peptides. Even though limited to glutamic- and aspartic acid residues, this study provides 1048 ADP-ribosylation sites from 340 proteins that cover also various functions in DNA metabolism as well as, for example, mRNA processing.

As ADP-ribosylation is very transient and difficult to detect, both studies (119, 141) mentioned above made use of siPARG or shPARG cell lines to boost the cellular ADP-ribosylation signal during the different stimulations. So far, the impact of PARG depletion on the cellular system has not been evaluated, but influences must be expected (e.g., different protein interactions due to hyper-ADP-ribosylation). Moreover, comparison of the two above-mentioned studies revealed that only a part of the modified peptides identified are the same, raising the question, whether the ADP-ribosylome is cell-type specific or whether the preparation influences the outcome. Moreover, it is very likely, that the two methods discussed have different affinities for mono- and poly-ADP-ribosylated proteins, and as a result, different subsets of the ADP-ribosylome have been identified. Therefore, improved and novel tools, as well as further extensive studies will be required to address the cellular ADP-ribosylome.

7. PERSPECTIVE

The research on the ADP-ribosylome so far clearly shows that different classes of amino acids on a broad variety of target proteins are ADP-ribosylated. Furthermore, depending on the analyzed conditions, modifications can comprise single ADP-ribose moieties or PAR chains of various lengths and branching patterns. Due to this huge complexity in acceptor proteins, modification sites, linkage types and ADP-ribose structures, it is highly unlikely that one method alone will be able to reliably detect all ADP-ribosylated proteins in a given sample, e.g. a cell lysate. In the future, more large-scale systematic studies and new approaches are needed to build a comprehensive database of all ADP-ribose modifications. It would be highly advantageous if the research community agreed on a limited number of cell types to allow comparison of the results obtained by different methods and under different experimental conditions.

7.1. Identification of a conserved ADP-ribosylation motif

A comprehensive database of identified and confirmed ADP-ribose acceptor sites will not only serve as a foundation for in-depth analyses of the cellular function of ADP-ribose modification, but may also allow the identification of a consensus sequence for ADP-ribosylation sites. Such an ADP-ribosylation motif could become an important tool to predict all potential ADP-ribosylation sites in different organisms and cells.

7.2. Functional contribution of the ARTD family members

One of the most challenging future tasks is the assignment of all ADP-ribose modifications (i.e., proteins and acceptor sites) to specific ARTD family members. Based on our current understanding, it is to be expected that the substrate specificities of the different ARTDs considerably overlap. Deletion or knockdown studies may therefore not be able to reveal the substrate spectrum of all ARTDs. To define which proteins are modified by each individual ARTD family member, *in vitro* experiments with recombinantly expressed ARTD proteins therefore seem to be the most promising approach. The results from such studies will help to predict the cellular contribution and function of all ARTD members, and therefore constitute the

foundation for their detailed and comprehensive analysis *in vivo.*

8. ACKNOWLEDGEMENT

We are grateful to Florian Freimoser, Stephan Christen and other members of the Institute of Veterinary Biochemistry and Molecular Biology (University of Zurich, Switzerland) for helpful advice and discussions. Research on ADP-ribosylation in the laboratory of MOH is supported by the Swiss National Science Foundation Grant 310030B_138667 and the Kanton of Zurich.

9. REFERENCES

1. O. N. Jensen: Interpreting the protein language using proteomics. *Nat Rev Mol Cell Biol*, 7(6), 391-403 (2006)

2. G. A. Khoury, R. C. Baliban and C. A. Floudas: Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Sci Rep*, 1 (2011)

3. E. S. Witze, W. M. Old, K. A. Resing and N. G. Ahn: Mapping protein post-translational modifications with mass spectrometry. *Nat Methods*, 4(10), 798-806 (2007)

4. W. L. Kraus and M. O. Hottiger: PARP-1 and gene regulation: Progress and puzzles. *Mol Aspects Med* (2013)

5. P. Chambon, J. D. Weill and P. Mandel: Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem Biophys Res Commun*, 11, 39-43 (1963)

6. M. O. Hottiger, P. O. Hassa, B. Lüscher, H. Schüler and F. Koch-Nolte: Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem Sci*, 35(4), 208-219 (2010)

7. R. Bredehorst, K. Wielckens, A. Gartemann, H. Lengyel, K. Klapproth and H. Hilz: Two different types of bonds linking single ADP-ribose residues covalently to proteins. Quantification in eukaryotic cells. *Eur J Biochem*, 92(1), 129-35 (1978)

8. A. Hakme, H. K. Wong, F. Dantzer and V. Schreiber: The expanding field of poly(ADP-ribosyl)ation reactions. 'Protein Modifications: Beyond the Usual Suspects' Review Series. *EMBO Rep*, 9(11), 1094-100 (2008)

9. R. Alvarez-Gonzalez and H. Mendoza-Alvarez: Dissection of ADP-ribose polymer synthesis into individual steps of initiation, elongation, and branching. *Biochimie*, 77(6), 403-7 (1995)

10. K. Wielckens, A. Schmidt, E. George, R. Bredehorst and H. Hilz: DNA fragmentation and NAD depletion. Their relation to the turnover of endogenous mono(ADP-ribosyl) and poly(ADP-ribosyl) proteins. *J Biol Chem*, 257(21), 12872-12877 (1982)

11. K. L. Feijs, P. Verheugd and B. Luscher: Expanding functions of intracellular resident mono-ADP-ribosylation in cell physiology. *FEBS J*, 280(15), 3519-3529 (2013)

12. A. K. Leung, S. Vyas, J. E. Rood, A. Bhutkar, P. A. Sharp and P. Chang: Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. *Mol Cell*, 42(4), 489-499 (2011)

13. X. Luo and W. L. Kraus: On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev*, 26(5), 417-432 (2012)

14. P. O. Hassa, S. Haenni, M. Elser and M. O. Hottiger: Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? *Microbiol Mol Biol Rev*, 70(3), 789-829 (2006)

15. V. Schreiber, F. Dantzer, J. C. Ame and G. de Murcia: Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mole Cell Biol*, 7(7), 517-28 (2006)

16. M. O. Hottiger: ADP-ribosylation of histones by ARTD1: An additional module of the histone code? *FEBS Lett*, 585, 1595-1599 (2011)

17. J. L. Riffell, C. J. Lord and A. Ashworth: Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. *Nat Rev Drug Discovery*, 11(12), 923-36 (2012)

18. F. Dantzer and R. Santoro: The expanding role of PARPs in the establishment and maintenance of heterochromatin. *FEBS J* (2013)

19. L. Virag, A. Robaszkiewicz, J. M. Vargas and F. Javier Oliver: Poly(ADP-ribose) signaling in cell death. *Mol Aspects Med* (2013)

20. C. T. Ong, K. Van Bortle, E. Ramos and V. G. Corces: Poly(ADP-ribosyl)ation regulates insulator function and intrachromosomal interactions in Drosophila. *Cell*, 155(1), 148-159 (2013)

21. P. F. Cho-Park and H. Steller: Proteasome regulation by ADP-ribosylation. *Cell*, 153(3), 614-627 (2013)

22. I. Welsby, D. Hutin and O. Leo: Complex roles of members of the ADP-ribosyl transferase super family in immune defences: looking beyond PARP1. *Biochem Pharmacol*, 84(1), 11-20 (2012)

23. B. A. Gibson and W. L. Kraus: New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat Rev Mol Cell Biol*, 13(7), 411-424 (2012)

24. R. Krishnakumar and W. L. Kraus: PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway. *Mol Cell*, 39(5), 736-49 (2010)

25. G. Asher, H. Reinke, M. Altmeyer, M. Gutierrez-Arcelus, M. O. Hottiger and U. Schibler: Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell*, 142(6), 943-953 (2010)

26. R. J. Fieldhouse and A. R. Merrill: Needle in the haystack: structure-based toxin discovery. *Trends Biochem Sci*, 33(11), 546-56 (2008)

27. Q. Deng and J. T. Barbieri: Molecular mechanisms of the cytotoxicity of ADP-ribosylating toxins. *Annu Rev Microbiol*, 62, 271-288 (2008)

28. Y. Shimizu, S. Hasegawa, S. Fujimura and T. Sugimura: Solubilization of enzyme forming ADPR polymer from NAD. *Biochem Biophys Res Commun*, 29(1), 80-3 (1967)

29. P. O. Hassa and M. O. Hottiger: The diverse biological roles of mammalian PARPs, a small but powerful family of poly-ADP-ribose polymerases. *Front Biosci*, 13, 3046-3082 (2008)

30. W. M. Shieh, J. C. Ame, M. V. Wilson, Z. Q. Wang, D. W. Koh, M. K. Jacobson and E. L. Jacobson: Poly(ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers. *J Biol Chem*, 273(46), 30069-72 (1998)

31. J. C. Ame, V. Rolli, V. Schreiber, C. Niedergang, F. Apiou, P. Decker, S. Muller, T. Hoger, J. Menissier-de Murcia and G. de Murcia: PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. *J Biol Chem*, 274(25), 17860-8 (1999)

32. O. Loseva, A. S. Jemth, H. E. Bryant, H. Schuler, L. Lehtio, T. Karlberg and T. Helleday: PARP-3 is a mono-ADP-ribosylase that activates PARP-1 in the absence of DNA. *J Biol Chem*, 285(11), 8054-60 (2010)

33. S. L. Rulten, A. E. Fisher, I. Robert, M. C. Zuma, M. Rouleau, L. Ju, G. Poirier, B. Reina-San-Martin and K. W. Caldecott: PARP-3 and APLF function together to accelerate nonhomologous end-joining. *Mol Cell*, 41(1), 33-45 (2011)

34. J. F. Rippmann, K. Damm and A. Schnapp: Functional characterization of the poly(ADP-ribose) polymerase activity of tankyrase 1, a potential regulator of telomere length. *J Mol Biol*, 323(2), 217-24 (2002)

35. B. D. Cook, J. N. Dynek, W. Chang, G. Shostak and S. Smith: Role for the related poly(ADP-Ribose) polymerases tankyrase 1 and 2 at human telomeres. *Mol Cell Biol*, 22(1), 332-42 (2002)

36. H. Kleine, E. Poreba, K. Lesniewicz, P. O. Hassa, M. O. Hottiger, D. W. Litchfield, B. H. Shilton and B. Lüscher: Substrate-assisted catalysis by PARP10 limits its activity to mono-ADP-ribosylation. *Mol Cell*, 32(1), 57-69 (2008)

37. R. C. Aguiar, K. Takeyama, C. He, K. Kreinbrink and M. A. Shipp: B-aggressive lymphoma family proteins have unique domains that modulate transcription and exhibit poly(ADP-ribose) polymerase activity. *J Biol Chem*, 280(40), 33756-33765 (2005)

38. S. Vyas, M. Chesarone-Cataldo, T. Todorova, Y. H. Huang and P. Chang: A systematic analysis of the PARP protein family identifies new functions critical for cell physiology. *Nat Commun*, 4, 2240 (2013)

39. M. De Vos, V. Schreiber and F. Dantzer: The diverse roles and clinical relevance of PARPs in DNA damage repair: Current state of the art. *Biochem Pharmacol*, 84(2), 137-46 (2012)

40. J. Krietsch, M. Rouleau, E. Pic, C. Ethier, T. M. Dawson, V. L. Dawson, J. Y. Masson, G. G. Poirier and J. P. Gagne: Reprogramming cellular events by poly(ADP-ribose)-binding proteins. *Mol Aspects Med* (2013)

41. E. Barkauskaite, G. Jankevicius, A. G. Ladurner, I. Ahel and G. Timinszky: The recognition and removal of cellular poly(ADP-ribose) signals. *FEBS J*, 280(15), 3491-3507 (2013)

42. J. M. Pleschke, H. E. Kleczkowska, M. Strohm and F. R. Althaus: Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. *J Biol Chem*, 275(52), 40974-80 (2000)

43. J. P. Gagne, M. Isabelle, K. S. Lo, S. Bourassa, M. J. Hendzel, V. L. Dawson, T. M. Dawson and G. G. Poirier: Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. *Nucleic Acids Res*, 36(22), 6959-76 (2008)

44. M. D. Allen, A. M. Buckle, S. C. Cordell, J. Lowe and M. Bycroft: The crystal structure of AF1521 a protein from Archaeoglobus fulgidus with homology to the non-histone domain of macroH2A. *J Mol Biol*, 330(3), 503-11 (2003)

45. J. R. Pehrson and V. A. Fried: MacroH2A, a core histone containing a large nonhistone region. *Science*, 257(5075), 1398-400 (1992)

46. T. Kalisch, J. C. Ame, F. Dantzer and V. Schreiber: New readers and interpretations of poly(ADP-ribosyl)ation. *Trends Biochem Sci*, 37(9), 381-90 (2012)

47. M. J. Gamble: Expanding the functional repertoire of macrodomains. *Nat Struct Mol Biol*, 20(4), 407-8 (2013)

48. A. H. Forst, T. Karlberg, N. Herzog, A. G. Thorsell, A. Gross, K. L Feijs, P. Verheugd, P. Kursula, B. Nijmeijer, E. Kremmer, H. Kleine, A. G. Ladurner, H. Schuler and B. Luscher: Recognition of mono-ADP-ribosylated ARTD10 substrates by ARTD8 macrodomains. *Structure*, 21(3), 462-475 (2013)

49. G. I. Karras, G. Kustatscher, H. R. Buhecha, M. D. Allen, C. Pugieux, F. Sait, M. Bycroft and A. G. Ladurner: The macro domain is an ADP-ribose binding module. *EMBO J*, 24(11), 1911-20 (2005)

50. M. Neuvonen and T. Ahola: Differential activities of cellular and viral macro domain proteins in binding of ADP-ribose metabolites. *J Mol Biol*, 385(1), 212-225 (2009)

51. M. P. Egloff, H. Malet, A. Putics, M. Heinonen, H. Dutartre, A. Frangeul, A. Gruez, V. Campanacci, C. Cambillau, J. Ziebuhr, T. Ahola and B. Canard: Structural and functional basis for ADP-ribose and poly(ADP-ribose) binding by viral macro domains. *J Virol*, 80(17), 8493-502 (2006)

52. S. Till and A. G. Ladurner: Sensing NAD metabolites through macro domains. *Front Biosci*, 14, 3246-58 (2009)

53. L. Aravind: The WWE domain: a common interaction module in protein ubiquitination and ADP ribosylation. *Trends Biochem Sci*, 26(5), 273-5 (2001)

54. Z. Wang, G. A. Michaud, Z. Cheng, Y. Zhang, T. R. Hinds, E. Fan, F. Cong and W. Xu: Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination. *Genes Dev*, 26(3), 235-240 (2012)

55. F. He, K. Tsuda, M. Takahashi, K. Kuwasako, T. Terada, M. Shirouzu, S. Watanabe, T. Kigawa, N. Kobayashi, P. Guntert, S. Yokoyama and Y. Muto: Structural insight into the interaction of ADP-ribose with the PARP WWE domains. *FEBS Lett*, 586(21), 3858-64 (2012)

56. J. Oberoi, M. W. Richards, S. Crumpler, N. Brown, J. Blagg and R. Bayliss: Structural basis of poly(ADP-ribose) recognition by the multizinc binding domain of checkpoint with forkhead-associated and RING Domains (CHFR). *J Biol Chem*, 285(50), 39348-58 (2010)

57. S. Eustermann, C. Brockmann, P. V. Mehrotra, J. C. Yang, D. Loakes, S. C. West, I. Ahel and D. Neuhaus: Solution structures of the two PBZ domains from human APLF and their interaction with poly(ADP-ribose). *Nat Struct Mol Biol*, 17(2), 241-3 (2010)

58. I. Ahel, D. Ahel, T. Matsusaka, A. J. Clark, J. Pines, S. J. Boulton and S. C. West: Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. *Nature*, 451(7174), 81-5 (2008)

59. L. L. Simpson: Molecular basis for the pharmacological actions of Clostridium botulinum type C2 toxin. *J Pharmacol Exp Ther*, 230(3), 665-9 (1984)

60. R. E. West, Jr., J. Moss, M. Vaughan, T. Liu and T. Y. Liu: Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site. *J Biol Chem*, 260(27), 14428-30 (1985)

61. J. Vandekerckhove, B. Schering, M. Barmann and K. Aktories: Botulinum C2 toxin ADP-ribosylates cytoplasmic beta/gamma-actin in arginine 177. *J Biol Chem*, 263(2), 696-700 (1988)

62. A. E. Lang, G. Schmidt, A. Schlosser, T. D. Hey, I. M. Larrinua, J. J. Sheets, H. G. Mannherz and K. Aktories: Photorhabdus luminescens toxins ADP-ribosylate actin and RhoA to force actin clustering. *Science*, 327(5969), 1139-42 (2010)

63. N. Ogata, K. Ueda, H. Kagamiyama and O. Hayaishi: ADP-ribosylation of histone H1. Identification of glutamic acid residues 2, 14, and the COOH-terminal lysine residue as modification sites. *J Biol Chem*, 255(16), 7616-20 (1980)

64. M. Kawaichi, K. Ueda and O. Hayaishi: Multiple autopoly(ADP-ribosyl)ation of rat liver poly(ADP-ribose) synthetase. Mode of modification and properties of automodified synthetase. *J Biol Chem*, 256(18), 9483-9 (1981)

65. K. Ueda and O. Hayaishi: ADP-ribosylation. *Annu Rev Biochem*, 54, 73-100 (1985)

66. J. Moss and M. Vaughan: Isolation of an avian erythrocyte protein possessing ADP-ribosyltransferase activity and capable of activating adenylate cyclase. *Proc Natl Acad Sci USA*, 75(8), 3621-4 (1978)

67. D. A. Yost and J. Moss: Amino acid-specific ADP-ribosylation. Evidence for two distinct NAD:arginine ADP-ribosyltransferases in turkey erythrocytes. *J Biol Chem*, 258(8), 4926-9 (1983)

68. F. Rosenthal, K. L. H. Feijs, E. Frugier, M. Bonalli, A. H. Forst, R. Imhof, H. C. Winkler, D. Fischer, A. Caflisch, P. O. Hassa, B. Lüscher and M. O. Hottiger: Macrodomain-containing proteins are novel mono-ADP-ribosylhydrolases. *Nat Struct Mol Biol*, 20(4), 502-507 (2013)

69. M. Altmeyer, S. Messner, P. O. Hassa, M. Fey and M. O. Hottiger: Molecular mechanism of poly(ADP-ribosyl)ation by PARP1 and identification of lysine residues as ADP-ribose acceptor sites. *Nucleic Acids Res*, 37(11), 3723-3738 (2009)

70. N. J. Oppenheimer: Structural determination and stereospecificity of the choleragen-catalyzed reaction of NAD+ with guanidines. *J Biol Chem*, 253(14), 4907-10 (1978)

71. D. Cervantes-Laurean, E. L. Jacobson and M. K. Jacobson: Glycation and glycoxidation of histones by ADP-ribose. *J Biol Chem*, 271(18), 10461-9 (1996)

72. E. Kun, A. C. Chang, M. L. Sharma, A. M. Ferro and D. Nitecki: Covalent modification of proteins by metabolites of NAD+. *Proc Natl Acad Sci USA*, 73(9), 3131-5 (1976)

73. D. Cervantes-Laurean, P. T. Loflin, D. E. Minter, E. L. Jacobson and M. K. Jacobson: Protein modification by ADP-ribose via acid-labile linkages. *J Biol Chem*, 270(14), 7929-36 (1995)

74. D. Cervantes-Laurean, D. E. Minter, E. L. Jacobson and M. K. Jacobson: Protein glycation by ADP-ribose: studies of model conjugates. *Biochemistry*, 32(6), 1528-34 (1993)

75. R. Alvarez-Gonzalez and F. R. Althaus: Poly(ADP-ribose) catabolism in mammalian cells exposed to DNA-damaging agents. *Mutat Res*, 218(2), 67-74 (1989)

76. G. Brochu, G. M. Shah and G. G. Poirier: Purification of poly(ADP-ribose) glycohydrolase and detection of its isoforms by a zymogram following one- or two-dimensional electrophoresis. *Anal Biochem*, 218(2), 265-72 (1994)

77. M. L. Meyer-Ficca, R. G. Meyer, D. L. Coyle, E. L. Jacobson and M. K. Jacobson: Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp Cell Res*, 297(2), 521-32 (2004)

78. W. Min and Z. Q. Wang: Poly (ADP-ribose) glycohydrolase (PARG) and its therapeutic potential. *Front Biosci (Landmark Ed)*, 14, 1619-26 (2009)

79. D. Slade, M. S. Dunstan, E. Barkauskaite, R. Weston, P. Lafite, N. Dixon, M. Ahel, D. Leys and I. Ahel: The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature*, 477(7366), 616-20 (2011)

80. C. N. Patel, D. W. Koh, M. K. Jacobson and M. A. Oliveira: Identification of three critical acidic residues of poly(ADP-ribose) glycohydrolase involved in catalysis: determining the PARG catalytic domain. *Biochem J*, 388(Pt 2), 493-500 (2005)

81. E. Barkauskaite, A. Brassington, E. S. Tan, J. Warwicker, M. S. Dunstan, B. Banos, P. Lafite, M. Ahel, T. J. Mitchison, I. Ahel and D. Leys: Visualization of poly(ADP-ribose) bound to PARG reveals inherent balance between exo- and endo-glycohydrolase activities. *Nat Commun*, 4, 2164 (2013)

82. G. Brochu, C. Duchaine, L. Thibeault, J. Lagueux, G. M. Shah and G. G. Poirier: Mode of action of poly(ADP-ribose) glycohydrolase. *Biochim Biophys Acta*, 1219(2), 342-50 (1994)

83. S. A. Braun, P. L. Panzeter, M. A. Collinge and F. R. Althaus: Endoglycosidic cleavage of branched polymers by poly(ADP-ribose) glycohydrolase. *Eur J Biochem*, 220(2), 369-375 (1994)

84. S. Oka, J. Kato and J. Moss: Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. *J Biol Chem*, 281(2), 705-13 (2006)

85. C. Mueller-Dieckmann, S. Kernstock, M. Lisurek, J. P. von Kries, F. Haag, M. S. Weiss and F. Koch-Nolte: The structure of human ADP-ribosylhydrolase 3 (ARH3) provides insights into the reversibility of protein ADP-ribosylation. *Proc Natl Acad Sci USA*, 103(41), 15026-31 (2006)

86. G. Glowacki, R. Braren, K. Firner, M. Nissen, M. Kuhl, P. Reche, F. Bazan, M. Cetkovic-Cvrlje, E. Leiter, F. Haag and F. Koch-Nolte: The family of toxin-related ecto-ADP-ribosyltransferases in humans and the mouse. *Protein Sci*, 11(7), 1657-70 (2002)

87. A. G. McLennan: The Nudix hydrolase superfamily. *Cell Mol Life Sci*, 63(2), 123-43 (2006)

88. E. Kraszewska: The plant Nudix hydrolase family. *Acta Biochim Pol*, 55(4), 663-71 (2008)

89. L. Gasmi, J. L. Cartwright and A. G. McLennan: Cloning, expression and characterization of YSA1H, a human adenosine 5'-diphosphosugar pyrophosphatase possessing a MutT motif. *Biochem J*, 344 Pt 2, 331-7 (1999)

90. S. Lin, L. Gasmi, Y. Xie, K. Ying, S. Gu, Z. Wang, H. Jin, Y. Chao, C. Wu, Z. Zhou, R. Tang, Y. Mao and A. G. McLennan: Cloning, expression and characterisation of a human Nudix hydrolase specific for adenosine 5'-diphosphoribose (ADP-ribose). *Biochim Biophys Acta*, 1594(1), 127-35 (2002)

91. A. Carloto, M. J. Costas, J. C. Cameselle, A. G. McLennan and J. M. Ribeiro: The specific, submicromolar-Km ADP-ribose pyrophosphatase purified from human placenta is enzymically indistinguishable from recombinant NUDT9 protein, including a selectivity for Mn2+ as activating cation and increase in Km for ADP-ribose, both elicited by H2O2. *Biochim Biophys Acta*, 1760(10), 1545-1551 (2006)

92. J. Moss, S. J. Stanley, M. S. Nightingale, J. J. Murtagh, Jr., L. Monaco, K. Mishima, H. C. Chen, K. C. Williamson and S. C. Tsai: Molecular and immunological characterization of ADP-ribosylarginine hydrolases. *J Biol Chem*, 267(15), 10481-8 (1992)

93. I. J. Okazaki, A. Zolkiewska, M. S. Nightingale and J. Moss: Immunological and structural conservation of mammalian skeletal muscle glycosylphosphatidylinositol-linked ADP-ribosyltransferases. *Biochemistry*, 33(43), 12828-36 (1994)

94. R. Braren, G. Glowacki, M. Nissen, F. Haag and F. Koch-Nolte: Molecular characterization and expression of the gene for mouse NAD+:arginine ecto-mono(ADP-ribosyl)transferase, Art1. *Biochem J*, 336 (Pt 3), 561-8 (1998)

95. J. Kato, J. Zhu, C. Liu, M. Stylianou, V. Hoffmann, M. J. Lizak, C. G. Glasgow and J. Moss: ADP-ribosylarginine hydrolase regulates cell proliferation and tumorigenesis. *Cancer Res*, 71(15), 5327-35 (2011)

96. F. C. Peterson, D. Chen, B. L. Lytle, M. N. Rossi, I. Ahel, J. M. Denu and B. F. Volkman: Orphan macrodomain protein (human C6orf130) is an O-acyl-

ADP-ribose deacylase: solution structure and catalytic properties. *J Biol Chem*, 286(41), 35955-35965 (2011)

97. D. Chen, M. Vollmar, M. N. Rossi, C. Phillips, R. Kraehenbuehl, D. Slade, P. V. Mehrotra, F. von Delft, S. K. Crosthwaite, O. Gileadi, J. M. Denu and I. Ahel: Identification of macrodomain proteins as novel O-acetyl-ADP-ribose deacetylases. *J Biol Chem*, 286(15), 13261-13271 (2011)

98. K. G. Tanner, J. Landry, R. Sternglanz and J. M. Denu: Silent information regulator 2 family of NAD- dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose. *Proc Natl Acad Sci USA*, 97(26), 14178-82 (2000)

99. G. Jankevicius, M. Hassler, B. Golia, V. Rybin, M. Zacharias, G. Timinszky and A. G. Ladurner: A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. *Nat Struct Mol Biol*, 20(4), 508-514 (2013)

100. K. L. Feijs, A. H. Forst, P. Verheugd and B. Luscher: Macrodomain-containing proteins: regulating new intracellular functions of mono(ADP-ribosyl)ation. *Nat Rev Mol Cell Biol*, 14(7), 443-451 (2013)

101. R. Sharifi, R. Morra, C. Denise Appel, M. Tallis, B. Chioza, G. Jankevicius, M. A. Simpson, I. Matic, E. Ozkan, B. Golia, M. J. Schellenberg, R. Weston, J. G. Williams, M. N. Rossi, H. Galehdari, J. Krahn, A. Wan, R. C. Trembath, A. H. Crosby, D. Ahel, R. Hay, A. G. Ladurner, G. Timinszky, R. S. Williams and I. Ahel: Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. *EMBO J*, 32(9), 1225-1237 (2013)

102. L. Meng, G. A. Michaud, J. S. Merkel, F. Zhou, J. Huang, D. R. Mattoon and B. Schweitzer: Protein kinase substrate identification on functional protein arrays. *BMC Biotech*, 8, 22 (2008)

103. K. L. Feijs, H. Kleine, A. Braczynski, A. H. Forst, N. Herzog, P. Verheugd, U. Linzen, E. Kremmer and B. Luscher: ARTD10 substrate identification on protein microarrays: regulation of GSK3beta by mono-ADP-ribosylation. *Cell Commun Signal*, 11(1), 5 (2013)

104. S. Troiani, R. Lupi, R. Perego, S. R. Depaolini, S. Thieffine, R. Bosotti and L. Rusconi: Identification of candidate substrates for poly(ADP-ribose) polymerase-2 (PARP2) in the absence of DNA damage using high-density protein microarrays. *FEBS J*, 278(19), 3676-3687 (2011)

105. J. Fila and D. Honys: Enrichment techniques employed in phosphoproteomics. *Amino Acids*, 43(3), 1025-47 (2012)

106. B. Bodenmiller and R. Aebersold: Quantitative analysis of protein phosphorylation on a system-wide scale by mass spectrometry-based proteomics. *Methods Enzymol*, 470, 317-34 (2010)

107. H. Jiang, J. H. Kim, K. M. Frizzell, W. L. Kraus and H. Lin: Clickable NAD analogues for labeling substrate proteins of poly(ADP-ribose) polymerases. *J Am Chem Soc*, 132(27), 9363-72 (2010)

108. H. Okayama, K. Ueda and O. Hayaishi: Purification of ADP-ribosylated nuclear proteins by covalent chromatography on dihydroxyboryl polyacrylamide beads and their characterization. *Proc Natl Acad Sci USA*, 75(3), 1111-5 (1978)

109. P. Adamietz, K. Klapproth and H. Hilz: Isolation and partial characterization of the ADP-ribosylated nuclear proteins from Ehrlich ascites tumor cells. *Biochem Biophys Res Commun*, 91(4), 1232-1238 (1979)

110. H. L. Weith, J. L. Wiebers and P. T. Gilham: Synthesis of cellulose derivatives containing the dihydroxyboryl group and a study of their capacity to form specific complexes with sugars and nucleic acid components. *Biochemistry*, 9(22), 4396-4401 (1970)

111. L. A. Trivelli, H. M. Ranney and H. T. Lai: Hemoglobin components in patients with diabetes mellitus. *N Engl J Med*, 284(7), 353-357 (1971)

112. F. Rosenthal, S. Messner, B. Roschitzki, P. Gehrig, P. Nanni and M. O. Hottiger: Identification of distinct amino acids as ADP-ribose acceptor sites by mass spectrometry. *Methods Mol Biol*, 780, 57-66 (2011)

113. A. Takatsy, K. Boddi, L. Nagy, G. Nagy, S. Szabo, L. Marko, I. Wittmann, R. Ohmacht, T. Ringer, G. K. Bonn, D. Gjerde and Z. Szabo: Enrichment of Amadori products derived from the nonenzymatic glycation of proteins using microscale boronate affinity chromatography. *Anal Biochem*, 393(1), 8-22 (2009)

114. D. Tsikas: Affinity chromatography as a method for sample preparation in gas chromatography/mass spectrometry. *J Biochem Biophys Methods*, 49(1-3), 705-731 (2001)

115. L. Ren, Z. Liu, Y. Liu, P. Dou and H. Y. Chen: Ring-opening polymerization with synergistic co-monomers: access to a boronate-functionalized polymeric monolith for the specific capture of cis-diol-containing biomolecules under neutral conditions. *Angew Chem Int Ed Engl*, 48(36), 6704-6707 (2009)

116. Y. Liu, L. Ren and Z. Liu: A unique boronic acid functionalized monolithic capillary for specific capture, separation and immobilization of cis-diol biomolecules. *Chem Commun (Camb)*, 47(17), 5067-5069 (2011)

117. H. Kawamitsu, H. Hoshino, H. Okada, M. Miwa, H. Momoi and T. Sugimura: Monoclonal antibodies to poly(adenosine diphosphate ribose) recognize different structures. *Biochemistry*, 23(16), 3771-7 (1984)

118. N. Dani, A. Stilla, A. Marchegiani, A. Tamburro, S. Till, A. Ladurner, D. Corda and M. Di Girolamo: Combining affinity purification by ADP-ribose-binding macro domains

with mass spectrometry to define the mammalian ADP-ribosyl proteome. *Proc Natl Acad Sci USA*, 106(11), 4243-8 (2009)

119. S. Jungmichel, F. Rosenthal, M. Altmeyer, J. Lukas, M. O. Hottiger and M. L. Nielsen: Proteome-wide identification of poly(ADP-ribosyl)ation targets in different genotoxic stress responses. *Mol Cell, 52(2), 272-85* (2013)

120. N. Dani, A. Stilla, A. Marchegiani, A. Tamburro, S. Till, A. G. Ladurner, D. Corda and M. Di Girolamo: Combining affinity purification by ADP-ribose-binding macro domains with mass spectrometry to define the mammalian ADP-ribosyl proteome. *Proc Natl Acad Sci USA*, 106(11), 4243-8 (2009)

121. N. L. Young, M. D. Plazas-Mayorca and B. A. Garcia: Systems-wide proteomic characterization of combinatorial post-translational modification patterns. *Expert Rev Proteomics*, 7(1), 79-92 (2010)

122. A. Bensimon, A. J. Heck and R. Aebersold: Mass spectrometry-based proteomics and network biology. *Annu Rev Biochem*, 81, 379-405 (2012)

123. K. Engholm-Keller and M. R. Larsen: Technologies and challenges in large-scale phosphoproteomics. *Proteomics*, 13(6), 910-931 (2013)

124. K. B. Sylvestersen, C. Young and M. L. Nielsen: Advances in characterizing ubiquitylation sites by mass spectrometry. *Curr Opin Chem Biol*, 17(1), 49-58 (2013)

125. H. Desaire: Glycopeptide analysis, recent developments and applications. *Mol Cell Proteomics*, 12(4), 893-901 (2013)

126. S. M. Margarit, W. Davidson, L. Frego and C. E. Stebbins: A steric antagonism of actin polymerization by a salmonella virulence protein. *Structure*, 14(8), 1219-29 (2006)

127. S. M. Hengel, S. A. Shaffer, B. L. Nunn and D. R. Goodlett: Tandem mass spectrometry investigation of ADP-ribosylated kemptide. *J Am Soc Mass Spectrom*, 20(3), 477-483 (2009)

128. R. Bakhtiar and Z. Guan: Electron capture dissociation mass spectrometry in characterization of peptides and proteins. *Biotechnol Lett*, 28(14), 1047-59 (2006)

129. K. Breuker, H. Oh, C. Lin, B. K. Carpenter and F. W. McLafferty: Nonergodic and conformational control of the electron capture dissociation of protein cations. *Proc Natl Acad Sci USA*, 101(39), 14011-6 (2004)

130. L. M. Mikesh, B. Ueberheide, A. Chi, J. J. Coon, J. E. Syka, J. Shabanowitz and D. F. Hunt: The utility of ETD mass spectrometry in proteomic analysis. *Biochim Biophys Acta*, 1764(12), 1811-22 (2006)

131. B. M. Zee and B. A. Garcia: Electron transfer dissociation facilitates sequencing of adenosine diphosphate-ribosylated peptides. *Anal Chem*, 82(1), 28-31 (2010)

132. M. Fedorova, A. Frolov and R. Hoffmann: Fragmentation behavior of Amadori-peptides obtained by non-enzymatic glycosylation of lysine residues with ADP-ribose in tandem mass spectrometry. *J Mass Spectrom*, 45(6), 664-9 (2010)

133. S. Messner, M. Altmeyer, H. Zhao, A. Pozivil, B. Roschitzki, P. Gehrig, D. Rutishauser, D. Huang, A. Caflisch and M. O. Hottiger: PARP1 ADP-ribosylates lysine residues of the core histone tails. *Nucleic Acids Res*, 38(19), 6350-6362 (2010)

134. J. V. Olsen, B. Macek, O. Lange, A. Makarov, S. Horning and M. Mann: Higher-energy C-trap dissociation for peptide modification analysis. *Nat Methods*, 4(9), 709-12 (2007)

135. M. Mann and N. L. Kelleher: Precision proteomics: the case for high resolution and high mass accuracy. *Proc Natl Acad Sci USA*, 105(47), 18132-8 (2008)

136. M. P. Jedrychowski, E. L. Huttlin, W. Haas, M. E. Sowa, R. Rad and S. P. Gygi: Evaluation of HCD- and CID-type fragmentation within their respective detection platforms for murine phosphoproteomics. *Mol Cell Proteomics*, 10(12), M111 009910 (2011)

137. J. D. Chapman, J. P. Gagne, G. G. Poirier and D. R. Goodlett: Mapping PARP-1 auto-ADP-ribosylation sites by liquid chromatography-tandem mass spectrometry. *J Proteome Res* (2013)

138. V. Rolli, M. O'Farrell, J. Menissier-de Murcia and G. de Murcia: Random mutagenesis of the poly(ADP-ribose) polymerase catalytic domain reveals amino acids involved in polymer branching. *Biochemistry*, 36(40), 12147-54 (1997)

139. G. T. Marsischky, B. A. Wilson and R. J. Collier: Role of glutamic acid 988 of human poly-ADP-ribose polymerase in polymer formation. Evidence for active site similarities to the ADP-ribosylating toxins. *J Biol Chem*, 270(7), 3247-54 (1995)

140. Z. Tao, P. Gao and H. Liu: Identification of the ADP-ribosylation sites in the PARP-1 automodification domain: analysis and implications. *J Am Chem Soc*, 131(40), 14258-14260 (2009)

141. Y. Zhang, J. Wang, M. Ding and Y. Yu: Site-specific characterization of the Asp- and Glu-ADP-ribosylated proteome. *Nat Methods*, 10(10), 981-4 (2013)

142. R. Alvarez-Gonzalez, H. Juarez-Salinas, E. L. Jacobson and M. K. Jacobson: Evaluation of immobilized boronates for studies of adenine and

pyridine nucleotide metabolism. *Anal Biochem*, 135(1), 69-77 (1983)

143. J. Moss, D. A. Yost and S. J. Stanley: Amino acid-specific ADP-ribosylation. *J Biol Chem*, 258(10), 6466-70 (1983)

Key Words: NAD^+, ADP-ribosylation, ARTD, PARP, Acceptor Side, Mass Spectrometry, Post-Translational Modification, Activity, Review

Send correspondence to: Michael O. Hottiger, Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland, Tel: 41 44-635-5474, Fax: 41 44-635-6840, E-mail: hottiger@vetbio.uzh.ch