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STATE-OF-THE-ART REVIEW

ADP-ribosylation: new facets of an ancient modification

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Rapid response to environmental changes is achieved by uni- and multicellular organisms through a series of molecular events, often involving modification of macromolecules, including proteins, nucleic acids and lipids. Amongst these, ADP-ribosylation is of emerging interest because of its ability to modify different macromolecules in the cells, and its association with many key biological processes, such as DNA-damage repair, DNA replication, transcription, cell division, signal transduction, stress and infection responses, microbial pathogenicity and aging. In this review, we provide an update on novel pathways and mechanisms regulated by ADP-ribosylation in organisms coming from all kingdoms of life.

Introduction

Evolution shows remarkable examples of how living species adapt and survive in response to natural and environmental changes [1]. All living organisms have evolved molecular mechanisms that enable them to quickly adapt to nutritional, chemical or physical alterations. These adaptations are induced by cascades of molecular events involving qualitative and quantitative changes in the basic, cellular macromolecules, such as proteins, nucleic acids and lipids. Ultimately, these signalling events will trigger the appropriate response. One of the most common tools to induce a rapid change in the cellular environment is the post-translational modification (PTM) of proteins by addition of chemical moieties, such as phosphate, acyl (most commonly methyl and acetate), small proteins or sugars [2]. One highly conserved PTM system is the ADP-ribosylation, the addition of ADP-ribose (ADPr) groups from nicotinamide adenine dinucleotide (NAD^+) to proteins [3] (Fig. 1). Interestingly, ADP-ribosylation can happen not only on proteins but also on other macromolecules such as DNA, or small chemical groups [4–7] (Fig. 1). The first discovered ADP-ribosyltransferase (ART) enzymes were identified as bacterial toxins, such as the Cholera and Diphtheria toxins [7,8]. These toxins are released from bacterial pathogens to irreversibly modify host proteins to gain an advantage over the infected host [7–9]. Later on, homologous transferases and modification-reversing hydrolytic enzymes have been

Abbreviations
ADPr, ADP-ribose; ART, ADP-ribosyltransferase; ARTC, Cholera toxin-like ART; ARTD, Diphtheria toxin-like ART; MARylation, mono(ADP-ribosyl)ation; monoARTs, mono(ADP-ribosyl)transferases; NAD^+, nicotinamide adenine dinucleotide; NUDIX, Nucleoside Diphosphate linked to X-moiety hydrolases; *O*AADPr, *O*-acetyl-ADP-ribose; PARG, poly(ADP-ribose) glycohydrolase; PAR, poly-ADP-ribose; PARP, poly(ADP-ribose) polymerase; PARylation, poly(ADP-ribosyl)ation; PR, phosphoribosylation; PTM, post-translational modification.

discovered in organisms from all kingdoms of life [3]. Moreover, many recent observations show how viral genomes evolved the genetic tools that enable them to modulate ADP-ribosylation signalling of infected cells [10–13]. ADP-ribosylation seems to be particularly prominent in the highest organisms and it is best studied for the poly(ADP-ribose) polymerase (PARP) superfamily of ART enzymes [3,7,14–16]. Altogether, ADP-ribosylation is a widespread modification that controls a vast number of cellular processes, including DNA damage repair, transcription, cell-cycle progression, cell division, unfolded protein response, aging, nitrogen fixation, microbial pathogenicity, cell death and many others [7,14,17–30]. However, our understanding of ADP-ribosylation is still in its infancy, as can be seen from the current rapid rate of discoveries of previously unknown pathways regulated by ADP-ribosylation.

ADP-ribosyltransferases

All so far characterized ARTs use NAD^+ cofactor and transfer a single or multiple ADPr moieties onto an acceptor molecule (termed mono- and poly(ADP-ribosyl)ation, also called MARylation and PARylation respectively) combined with the release of nicotinamide (NA; Fig. 1) [3,7,16]. The most widespread super families of ARTs contain transferase folds evolutionary related to bacterial toxins. These proteins can be grouped into: (a) PARP-like proteins, alternatively called Diphtheria toxin-like ART (ARTD) superfamily; and (b) Cholera toxin-like ART (ARTCs) superfamily. Most of the transferases from these groups are known to modify proteins, however, some of them modify DNA or small chemical groups such as phosphate [3–9,16,17,31] (Fig. 1).

Eighteen mammalian genes with a sequence homology to PARPs/ARTDs have been described [3,7,14,16,27,28]. The first and best characterized of these proteins was originally noted for its ability to synthesize ADPr polymers upon DNA damage and called poly(ADP-ribose) polymerase 1 (PARP1) [15,23,26,32–34]. Human PARP1, PARP2 and Tankyrases (PARP5a and PARP5b), and close homologues from lower organisms and bacteria are able to

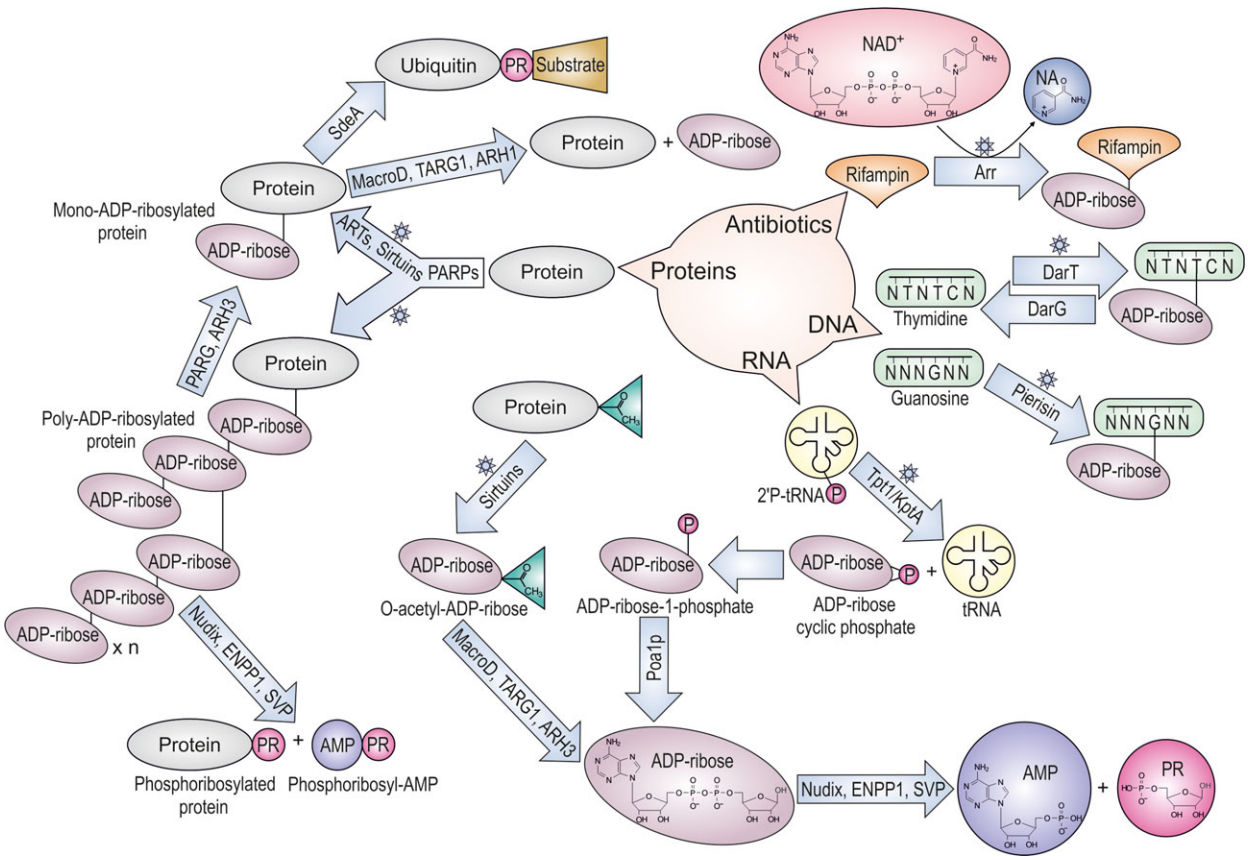

Fig. 1. Targets and pathways involved in the metabolism of ADP-ribose. Scheme is simplified to show only the main products and ADP-ribose metabolites. Stars indicate reactions that use NAD^+. PR, ribose-5′-phosphate.

produce long repeating chains of ADPr on target proteins [3,7,16,23,35–37]. PARP1 and PARP2 can also catalyse the formation of branched chains of poly (ADP-ribose) (PAR) on proteins and possibly DNA [6,35]. Most of the other characterized PARPs are mono(ADP-ribosyl) transferases (monoARTs) [16,24,35]. Another, highly diverged ART may also belong to PARP-like proteins, but it transfers a phosphate group from RNA onto ADPr (Fig. 1). This is KptA/Tpt1 protein, RNA phosphotransferase found in all three domains of life [7,38]. In yeast, Tpt1 catalyses a NAD^+-dependent dephosphorylation of tRNA-splicing intermediates, generating ADPr-1-phopshate through a cyclic intermediate [38] (Fig. 1).

Among the ARTC subfamily of transferases, pierisins are the only transferases able to act on DNA [4], all the other enzymes in this group characterised so far act as transferases for proteins [7,17]. Several bacterial toxins can be included in this family, such as the C3 ectotoxin from *Staphylococcus aureus*, VIP2 from *Bacillus cereus*, and SpvB from *Salmonella typhimurium* [7,39–41]. Mammalian ARTC family includes four human proteins (hARTC1, 3, 4, 5) that are glycosylphosphatidylinositol (GPI)-anchored or secreted proteins [17,42]. hARTCs have been reported to modify soluble and plasma membrane-associated protein targets and thus they are proposed to be involved in intercellular signalling, immune responses and inflammation [17,42,43].

Evolutionary unrelated ART enzymes to the previous group are sirtuins. Sirtuins are best known as NAD^+-dependent protein deacetylaeses and they are found in proteins of all kingdoms of life [44,45] (Fig. 1). There are seven sirtuin proteins operating in human cells [46], their primary enzymatic activity is protein deacetylation producing *O*-acetyl-ADP-ribose (*O*AADPr) metabolite as a by-product of its ART reaction [47]. Sirtuins can sometimes directly modify proteins [48,49] (Fig. 1).

It has been suggested that the nonenzymatic ADP-ribosylation of proteins may reach significant levels *in vivo*. This is due to the chemical reactivity of the free ADPr with side chains of variety of amino acids, most notably lysine and cysteine [50].

ADPr-binding domains

As with other PTMs, ADP-ribosylation tags are recognized by cellular proteins in a timely manner in order to activate downstream events in the relevant signalling pathways [16,26,30,51]. Therefore, many proteins involved in these pathways possess ADPr-binding domains within their protein structure [16,30,51]. Among the evolutionary widespread ADPr binding domains, the macrodomain has been studied the most extensively. Macrodomains are found in proteins from all kingdoms of life supporting different cellular processes [3,16,51–54]. Macrodomain-containing proteins can recognize variety of substrates, including MARylated and PARylated proteins, different ADPr metabolites (such as *O*AADPr) [16,53–55] and RNA [54,56,57] (Fig. 1). Some macrodomains have also evolved enzymatic activity and are capable of hydrolysing ADP-ribosylation (see below) [36,49,54,58,59]. As a consequence, macrodomain-containing proteins are involved in a diverse set of cellular functions, such as chromatin remodelling and DNA-damage repair, oxidative stress response, metabolic processes and pathogenic mechanisms [3,5,10–13,30,37,53,54,59–61]. In addition to macrodomain, several other widely distributed domains have been described as readers for ADP-ribosylation, such as the PAR-binding zinc finger (PBZ) [62], the WWE (named after three of its conserved residues) [63], the oligonucleotide/oligosaccharide-binding (OB) domain [64] and the PAR-binding motifs (PBM) which is abundant in DNA-damage repair proteins [65].

Hydrolases

As mentioned before, the ADP-ribosylation is a reversible modification [66]. Two evolutionary unrelated protein domains are known to support this catalytic activity: already mentioned macrodomains and the DraG-like fold containing proteins [3,67,68]. The catalytic macrodomain fold is found in a number of proteins coming from all the kingdoms of life [54]. In humans four catalytic macrodomains have been identified: poly(ADP-ribose) glycohydrolase (PARG), MacroD1, MacroD2 and terminal ADP-ribosyl glycohydrolase 1 (TARG1/C6orf130) [16,36,54,59,69,70]. PARG efficiently cleaves the PAR-specific *O*-glycosidic ribose–ribose bonds, however, it is unable to remove the terminal ADPr unit directly linked to a protein (Fig. 1) [36,58]. The existence of PARG-splicing variants ensure the presence of the enzyme both in the nucleus and in the cytoplasm or in membranous organelles [71–73] and allows for rapid turnover of PAR, ensuring tight control of this modification [66]. The terminal ADPr moiety is removed by MARylation preferring hydrolases, such as MacroD1, MacroD2 or TARG1 [58,59,69,70] (Fig. 1). The latter enzymes can also hydrolyse some other ADPr metabolites, such as *O*AADPr [54,55,74] (Fig. 1). An additional macrodomain containing protein is Poa1p (YBR022) from *Saccharomyces cerevisiae*, functionally characterized as a

specific phosphatase that removes the phosphate group of ADPr-1-phosphate in the tRNA-splicing pathway in yeast [75].

Another class of de-ADP-ribosylation enzymes includes the dinitrogenase reductase-activating glycohydrolase (DraG) and related proteins [67,68,73,76–79]. DraG is known to regulate, in conjunction with ART called DraT, the central enzyme of nitrogen fixation in several bacterial species [68,76]. Mammals carry distant homologues of DraG (called ARH1-3 in humans), whose functions are so far not fully understood. The ARH1 protein shows efficient hydrolytic activity against MARylated proteins on arginine residues [77]. ADP-ribosylated proteins on arginine are found on cellular plasma membrane, in the lumen of endoplasmic reticulum (ER) [42,43,78–81] and in cytoplasm [82,83]. ARH3 was shown to hydrolyse the *O*-glycosidic bond of PAR chains and *O*AADPr [79,84–86]. ARH2 is believed to be catalytically inactive [79,84,85].

The released ADPr by all the active hydrolases can be further recycled and eventually converted to ATP by enzymes such as members of the Nucleoside Diphosphate linked to X-moiety hydrolases (NUDIX) family [87–89].

Other enzymes that cleave protein ADP-ribosylation

Noncanonical enzymes able to perform the hydrolysis of ADPr linked to proteins have been recently identified. These include two unrelated protein families, the NUDIX [87,88] and Ectonucleotide Pyrophosphatase/Phosphodiesterase (ENPP) [90], both of which hydrolyse the ADPr phosphodiester bond in mono-ADP-ribose and PAR linked to proteins, thus liberating adenosine monophosphate (AMP) and phosphoribose-AMP and leaving ribose-5′-phosphate (phosphoribosylation; PR) tags bound to the protein [91–94] (Fig. 1). The NUDIX enzymes able to perform this reaction are human NUDT16 and *Escherichia coli* RppH [91,92]. Within the ENPP family of enzymes, vertebrate ENPP1 proteins and Phoshodiesterase I found in the poison glands of rattlesnakes (Snake Venom Phosphodiesterase) exhibit the same activity [93,94]. The physiological relevance of the activities of ENPP1 and NUDT16 enzymes against the protein ADP-ribosylation remains unclear.

Phosphoribosylation of proteins is also a consequence of the activity of Sde, an enzyme that couples the ART with a phosphodiesterase domain, associated with the control of the host ubiquitination signalling by human pathogen *Legionella pneumophila* (see detailed description below) [95,96].

Mammalian ADP-ribosylation signalling

ADP-ribosylation in mammals is known to regulate a number of different processes [14,17,24,26–28,30]. Best understood is regulation of DNA-damage repair pathways by PARP1-3 that are activated upon binding to DNA breaks [14,26–28,97–102] PARP1 also plays roles in transcription and metabolism [29,30,103]. The functions of other PARPs are comparatively much less understood [27,28]. PARP4 (also called VPARP) is a component of the cytosolic ribonucleoprotein vault complex, however, its biological functions are unknown [104]. PARPs 5a and 5b (tankyrases) are best understood for their roles in mitosis [19,25] and Wnt signalling [105–108], but they also have roles at telomere and DNA-damage repair [108–110]. PARP6 and PARP8 are poorly understood, however, PARP6 has been shown to be involved in hippocampus neuronal development [111]. Several PARPs (PARP7 PARP10, PARP12 and PARP13) are involved in mechanisms of post-transcriptional regulation of mRNA, mediated either by RNA-binding domains [112] or by ADP-ribosylation of RNA-binding proteins [20]. In addition, PARP10 has been implied in the regulation of NF-kB [113,114], GSK3β [113,115] and transcription [103,113,116]. Also, PARP9 and PARP14 are suggested to act on transcription, in particular of genes required for macrophage activation [117]. PARP16 regulates the unfolded protein response [18]. Concurrent with its nuclear pore localization, PARP11 modifies targets involved in the coordination of the nuclear envelope and the organization of nuclear pores [118,119].

Future work is needed to properly understand the physiological functions of most of the PARPs and the new potential functions for PARPs and other ARTs are continuously arising [120].

Compared to PARPs, much less research has been conducted on members of the ARTC family in mammals. This family includes four proteins in humans (hARTC1, 3, 4, 5) and six in mice (mARTC1, 2.1, 2.2, 3, 4, 5) that are glycosylphosphatidylinositol (GPI)-anchored (hARTC1 and mARTC1) or ecto-proteins (hARTC3, 4, 5 and mARCT2.1, 2.2, 3, 4, 5) [42]. mARTC1, 2, and 5 have been reported to modify soluble and plasma membrane-associated protein targets on arginine residues, including the P2X7 purinergic receptor, and thus they can affect cellular processes such as intercellular signalling, immune responses and inflammation [43]. hARTC1 has been detected in the lumen of ER and its function in stress response has been suggested [81].

Amino acid specificity of mammalian ARTs

Although all the ARTC proteins in mammals characterized so far modify substrate proteins on arginine residues [42], the situation for PARP family is more complex and there is still no strong consensus in the field on the preferred amino acid targets for many PARPs. In this respect, the progress has been additionally hampered in the case of poly(ADP-ribosyl)ating PARPs, as the current methods to identify sites do not make the difference between mono and poly (ADP-ribosyl)ation for specific amino acid position. Overall, acidic residues might be the main targets for most of the PARPs [35,121–124] but cysteine [35,120,123], arginine [121,123,124], lysine [83,121,123,124] and serine residues [125] have been suggested as well. ADP-ribosylation of acidic residues and lysines has been shown to be induced by oxidative stress [83]. However, additional evidence has revealed that many of these lysine residues may have been misannotated as modification sites, and that actual modification sites are proximal serine residues, that usually follow immediately after these lysine residues [126]. Indeed, the KS motif has been identified as a preferred target for serine ADP-ribosylation by several studies [125–127]. Notably, serine ADP-ribosylation seems to be specific for regulation of DNA damage response and other pathways important for genome stability such as regulation of chromatin structure, transcription and mitosis [127]. HPF1/C4orf27 is the first protein identified acting as a specificity factor for the serine ADP-ribosylation [127,128]. It acts in conjunction with PARP1 and PARP2 proteins and directs modification of histones, PARP1 itself, high-mobility group proteins and likely many other proteins [127].

ADP-ribosylation in bacteria

The first discovered ARTs were secreted toxins that are found sporadically in bacteria and that irreversibly modify crucial host cell proteins [129]. However, the genomic evidence suggests that intracellular, reversible ADP-ribosylation is much more common amongst bacteria, yet, there is little evidence on its physiological relevance. A notable exception is the DraT/DraG system of nitrogen-fixating bacteria from the *Azospirillum* and *Rhodospirillum* genera. DraT homologues are restricted to several nitrogen-fixing bacteria, while DraG homologues are distributed across all three domains of life [67]. Endogenous ADP-ribosylation has also been reported for some other bacterial species where this process probably regulates important cellular functions such as sporulation in *Bacillus subtilis* [130], development and cell–cell interaction in *Myxococcus xanthus* [131,132], as well as differentiation and secondary metabolism in *Streptomyces* [133–136].

Streptomyces – bacterial model organism for the study of ADP-ribosylation

So far, the most evidence for intracellular endogenous protein ADP-ribosylation has been found in *Streptomyces* species. *Streptomyces* are soil-inhabiting Gram-positive bacteria best known for their complex life cycle that includes morphological differentiation and the production of various secondary metabolites including antibiotics, anti-cancer drugs and immunosuppressors. ADP-ribosylation has been discovered in *Streptomyces* over 20 years ago [137]. First reports demonstrated considerable ADP-ribosylating activities in cell extracts and suggested a role for ADP-ribosylation in growth and differentiation processes in *Streptomyces griseus* [133,134]. In both *Str. griseus* and *Streptomyces coelicolor*, ADP-ribosylation patterns change with morphological differentiation [138,139] and several identified ADP-ribosylated proteins in *Str. coelicolor* suggested a connection between protein ADP-ribosylation and the regulation of metabolic requirements of the cells [135].

Streptomyces coelicolor genomic data (Table 1) suggest that ADP-ribosylation should be prominent in *Streptomyces*. Two ARTs have been characterized in *Streptomyces*, SCO5461 from *Str. coelicolor* [136,140] and Scabin from plant pathogen *Streptomyces scabies* [141]. These proteins are homologues of pierisins [4] and possess guanine-specific DNA ART activity, but

Table 1. Enzymes potentially involved in ADP-ribosylation process in *Streptomyces coelicolor*.

ADP-ribosyltransferases	SCO2860 (Arr homologue)
	SCO3953 (Tpt1/KptA homologue)
	SCO5461 (Pierisin homologue)
Macrodomain hydrolases	SCO0909 (bacterial-type PARG)
	SCO6450 (MacroD homologue)
	SCO6735 (TARG1-like)
DraG/ARH -like hydrolases	SCO0086
	SCO1766
	SCO2028
	SCO2029
	SCO2030
	SCO2031
	SCO4435
	SCO5809
Sirtuins	SCO0452
	SCO6464

they are not conserved across the *Streptomyces* species and cannot be found in *Str. griseus*, suggesting that the major protein ARTs in *Streptomyces* have yet to be discovered. Disruption of SCO5461 leads to conditional pleiotropic phenotype characterized by defects of morphological differentiation, antibiotic production and secretion [136].

The SCO3953 protein is a homologue of yeast tRNA 2′-phosphotransferase Tpt1, an essential enzyme in yeast that catalyses the final step in tRNA splicing. This reaction includes dephosphorylation of tRNA 2′-phosphate in two steps; transfer of ADPr from NAD^+ to tRNA 2′-phosphate that generates a 2′-phospho-ADPr-RNA intermediate and release of mature tRNA together with ADPr 1″-2″-cyclic phosphate [142] (Fig. 1). Tpt1 homologues are found distributed across all domains of life including bacterial species that have no known intron-containing tRNAs (*Str. coelicolor* and *E. coli* whose orthologue is called KptA are among them). Therefore, bacterial Tpt1/KptA homologues should have some yet uncovered substrate(s) and function(s).

The SCO2860 is a homologue of *Mycobacterium smegmatis* ART Arr that modifies antibiotic rifampicin (Fig. 1) and causes antibiotic resistance. *Mycobacterium smegmatis Arr* gene has been acquired from horizontal gene transfer [143]. It is upregulated after exposure to different kinds of stress and its endogenous cellular function has been proposed in a general stress response [144].

There is evidence of a much larger number of potential ADPr hydrolases in *Str. coelicolor*. Eight of them are uncharacterized DraG homologues and three are macrodomain proteins representing three different classes within the macrodomain superfamily (Table 1).

The SCO0909 is a bacterial-type PARG that cleaves the PARylation in the same manner as mammalian PARGs [36]. Nothing is known about the function of SCO0909, but in the radiation-resistant bacterium *Deinococcus radiodurans* the *Sco0909* gene is one of the most highly induced genes after DNA damage caused by ionizing radiation [145].

The SCO6450 is a MacroD homologue and it is predicted to remove protein MARylation. SCO6450 orthologues are found in most of the bacteria [54]. *Escherichia coli* homologue YmdB appears to be a multifunctional protein that regulates variety of cellular processes; deacetylates *O*AADPr, hydrolyses MARylated protein substrates, regulates RNAse III activity and modulates bacterial biofilm formation [55,69,146,147].

SCO6735 is a macrodomain protein closest to human proteins ALC1 and TARG1 [54,148]. *In vitro* SCO06735 can remove MARylation from glutamate residues, yet structural and biochemical characterization indicate a mechanism distinct from any other known macrodomain hydrolases [148]. Although SCO6735 physiological substrate is still unknown, its expression is under the control of a RecA-independent DNA damage inducible promoter [149,150] and upregulated upon UV-induced DNA damage [148], thus indicating a role in DNA damage response. Moreover, SCO6735 is possibly involved in the regulation of antibiotic production and disruption of the *Sco6735* gene was shown to increase actinorhodin production [148].

Two sirtuins, CobB1 (SCO0452) and CobB2 (SCO6464), have been identified in *Str. coelicolor*. CobB1 is a SIRT4 homologue that exhibits deacetylase activity on acetyl-CoA synthetase and consequently regulates its activity [151]. Auto-ADP-ribosylation was demonstrated for the SIRT4 homologue of *M. smegmatis* [152]. CobB2 appears to be related to SIRT5 and its overexpression suppresses production of two pigmented antibiotics, thus creating a loss-of-colouration phenotype [153].

Altogether, *Streptomyces* represent a good model for the study of ADP-ribosylation in bacteria and future studies on this model should help deciphering players and mechanisms of reversible ADP-ribosylation process. Since ADP-ribosylation is involved in the control of antibiotic production in *Streptomyces*, a better understanding of this process will also enable better exploitation of *Streptomyces* biotechnological potential.

Other notable ADP-ribosylation systems in bacteria

Studies looking either at the genomic context of ADP-ribosylating systems or their evolution in bacteria suggest that ADP-ribosylation might be involved in the regulation of many crucial cellular processes including bacterial persistence, oxidative stress response and adaptation to the host environment in general [5,9,49,154].

Reversible DNA ADP-ribosylation

A novel DNA-ribosylating toxin-antitoxin (TA) system has been identified in a variety of different bacterial species including the human pathogens *Mycobacterium tuberculosis* and enterohemorrhagic *E. coli* [5]. The toxin component of the TA system is a DNA ART (DarT), which catalyses the modification of the second thymidine base in the TNTC motif of ssDNA. This modification is reversed by the DNA ADPr glycohydrolase (DarG) activity of the antitoxin. The

substrate specificity of DarT led to the discovery that the ADP-ribosylation interferes with DNA replication and induces DNA damage signalling via the SOS response [5]. DarG belongs to the ALC1-like class of macrodomains and is structurally most similar to human TARG1. In addition to the reversal of the DNA ADP-ribosylation by DarG macrodomain hydrolytic activity, protein–protein interaction between DarT and DarG (resembling a type II TA system) revealed a second layer of DarT regulation [5]. All available data including the fact that DarG is essential in *M. tuberculosis* suggest that targeting this ADP-ribosylating TA system may have a therapeutic potential [5,155].

Sirtuin dependent ADP-ribosylation in regulation of oxidative stress

Protein ADP-ribosylation carried out by a distinct class of sirtuins (SirTM) was described in *Sta. aureus* and *Streptococcus pyogenes* and was suggested to regulate oxidative stress response in these pathogens. SirTM is encoded within an operon containing a modification carrier protein (GcvH-L). GcvH-L becomes doubly modified by two different PTMs through the actions of SirTM (ADP-ribosylation) and another component of the operon that acts as a lipoate ligase (synthesising the protein lipoylation) [2,49,156]. Yet another protein product of the same operon is a macrodomain protein (belonging to the MacroD-type class), which specifically reverses the ADP-ribosylation of GcvH-L [49]. It was suggested that the lipoylation acts as a scavenger of reactive oxygen species (either host derived or environmentally induced), while the reversible ADP-ribosylation may regulate interactions with other proteins involved in the oxidative stress response that are part of this protein complex [49]. SirTM homologues are found in a number of fungal pathogens [49].

ADP-ribosylation as precursor for ligase-independent ubiquitination

Bacterial-induced ADP-ribosylation has been implicated in regulation of host ubiquitination signalling, a eukaryotic-specific PTM via attachment of a small protein ubiquitin and associated with modulation of the target protein function or degradation [2,157]. The pathogenic bacterium *L. pneumophila* uses ubiquitin effector proteins of the Sde family, a new class of ubiquitin-specific monoART, to modulate the host ubiquitin signalling and create a favourable growth environment within the host cell [158]. One of the Sde proteins, SdeA, contains a monoART domain as well as phosphodiesterase domain (PDE) [95,96]. The monoART catalyses the ADP-ribosylation of ubiquitin on Arg42, while the PDE hydrolizes the ADPr-phosphodiester bond, thus establishing a 5′-phosphoribosyl modification (Fig. 1). Subsequently, the phosphoribosylated ubiquitin is linked to a serine residue within target proteins, thus completing an E2/3 ligase-independent ubiquitination system. Sde-mediated ubiquitination of several ER-associated Rab proteins and reticulon 4 impairs several cellular processes, such as mitophagy, TNF signalling, tubular endoplasmic reticulum functions and proteasomal degradation, allowing better bacterial growth [95,96].

ADP-ribosylation in archaea

Among archaea only the Tpt1/KptA ART type can be found widely spread. Considering its wide distribution in all three domains of life and structural simplicity, this protein could represent the ancestral version of the entire ART superfamily [9]. Other representatives of the ART superfamily can be found, but these are limited to only a few species per homologue [9]. In the two methane-producing archaea *Methanobrevibacter smithii* and *Methanospirillum hungatei* homologues of the exotoxin Alt/VIP2 were identified. A gene encoding a fusion-protein homologue of the DarT-DarG TA system was found in *Nitrosopumilus maritimus*. Protein ADP-ribosylating sirtuins (SirTM) have been found in the genomes of *Sulfolobus solfataricus* and *Methanobrevibacter* species [54,159]. Although PARPs-encoding genes could not been found in archaeal genomes, a PARP-like protein ADP-ribosylation activity has been detected in *Su. solfataricus* [160].

Enzymes capable of removing ADP-ribosylation are represented in archaea by two classes, MacroD-type and TARG1-like. The best-studied archaeal macrodomain protein is Af1521 from thermophile archaea *Arhaeoglobus fulgidus*. This protein is capable of binding both ADPr and PAR, and possesses enzymatic activity capable of hydrolysing Appr-1-P and MARylated protein substrates [52,69,70]. Af1521 is also used as a tool to enrich ADP-ribosylated protein for mass-spectrometry analyses of modification sites [83,161]. TARG1-like enzymes, as well as DraG homologues are sporadically found in some archaeal species such as *Methanococcus janaschii* (PDB code 1T5J).

ADP-ribosylation in viruses

Viruses can manipulate host ADP-ribosylation machinery and MARylation has been recognized as an

efficient weapon in the bacteriophage arsenal that is successfully used against bacterial antiphage defence [155,162]. Alt, ModA and ModB are T4 phage ARTC-like monoARTs that modify the *E. coli* host proteins shortly after infection to overtake the control of the host transcriptional and translational machinery. These enzymes together modify over 30 *E. coli* proteins, including RNA polymerase, ribosomal protein S1, EF-Tu and MazF [162,163]. Of these MazF belongs to one of the best-studied type II TA systems (MazE/MazF), which is involved in bacteriophage defence. MazE is a rapidly degraded antitoxin and MazF is a stable toxin with RNA cleavage activity (specific to ACA RNA sequence) that blocks protein synthesis. Using Alt, the T4 phage defends itself against this system by ADP-riboysilating MazF, impairing the RNA cleavage activity, and thus enables phage growth [163].

Another type of ARTs that can be identified in a limited number of dsDNA viruses are PARP-like proteins. These are most likely acquired by horizontal gene transfer and their physiological role remains yet to be studied [3].

Proteins encoding macrodomains are more frequently distributed in viral genomes and several different types of macrodomains can be found in both dsDNA and positive-strand ssRNA [10,54]. Viral macrodomains are usually part of larger proteins that contain additional domains. Biochemical, structural and phylogenetic evidences showed that viral and cellular macrodomains are closely related. Viral macrodomains bind ADPr and PAR and can perform activities characteristic for cellular macrodomains. Most viral macrodomains belong to MacroD-type class, but besides their basic de-MARylation activity, they are also capable to remove the whole PAR chain from PARylated substrates resembling TARG1 activity [10]. In coronaviruses, the MacroD-type macrodomain is a part of the multidomain nonstructural protein 3 (nsP3). It has been shown that this macrodomain promotes virulence and suppresses the innate immune response during severe acute respiratory syndrome (SARS) coronavirus infection [11]. In addition to the MacroD-type of macrodomains, a highly diverged macrodomain SUD-M was found as a part of the nsP3 in SARS coronaviruses. This unique macrodomain binds nucleic acids, preferentially RNA, and is crucial for viral genome replication/transcription [164]. Numerous other examples show that viral macrodomains affect virus replication and interferon-response in humans [12,13]. Viral macrodomains may act against mammalian PARPs that are known to possess antiviral activity [10]. PARPs involved in the antiviral defence are interferon-inducible, bear the signature of accelerated evolution and inhibit virus replication [165]. Specifically, PARP7, PARP10 and PARP12 have been experimentally shown to act as inhibitors of virus replication [166]. Another rapidly evolving PARP with broad antiviral activity is PARP13 (zinc finger antiviral protein), which specifically binds to viral RNA sequences targeting them for degradation [167]. Evidences for positive selection have also been found in macro-PARPs (PARP9, PARP14 and PARP15) and PARP4 [168]. In some cases, cellular PARP activity can be beneficial for viral infection rather than inhibitory [169,170].

Concluding remarks and future work

Numerous studies investigating ADP-ribosylation have been performed in the last several decades. Yet, our understanding of the molecular mechanisms governing ADP-ribosylation signalling and the physiological and pathophysiological importance of the pathways regulated by ADP-ribosylation are still poorly understood. Thus, there are many exciting findings waiting to be discovered in this field of research and the scientific community researching the ADP-ribosylation has been steadily growing in recent years. Many researchers are now also investing great efforts in developing new platforms, tools, methods and pipelines to study the ADP-ribosylation [83,91,122,123,125,161,171–175] these should greatly facilitate further understanding of the complexity of molecular and cellular mechanisms controlled by ADP-ribosylation.

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Author contributions

LP, AM and IA cowrote the manuscript and designed the figures.

Conflicts of interest

The authors have no conflicts of interest.

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