Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Review

Targeting ADP-ribosylation as an antimicrobial strategy

Giuliana Catara, Annunziata Corteggio, Carmen Valente, Giovanna Grimaldi, Luca Palazzo*

Institute of Protein Biochemistry, National Research Council of Italy, Via Pietro Castellino 111, Naples 80131, Italy

ARTICLE INFO

Keywords:

ADP-ribosylation
ADP-ribosyl transferase (ART)
ART bacterial toxins
Antimicrobial strategies
Poly(ADP-ribose) polymerase (PARP)

ABSTRACT

ADP-ribosylation (ADPr) is an ancient reversible modification of cellular macromolecules controlling major biological processes as diverse as DNA damage repair, transcriptional regulation, intracellular transport, immune and stress responses, cell survival and proliferation. Furthermore, enzymatic reactions of ADPr are central in the pathogenesis of many human diseases, including infectious conditions. By providing a review of ADPr signalling in bacterial systems, we highlight the relevance of this chemical modification in the pathogenesis of human diseases depending on host-pathogen interactions. The post-antibiotic era has raised the need to find alternative approaches to antibiotic administration, as major pathogens becoming resistant to antibiotics. An in-depth understanding of ADPr reactions provides the rationale for designing novel antimicrobial strategies for treatment of infectious diseases. In addition, the understanding of mechanisms of ADPr by bacterial virulence factors offers important hints to improve our knowledge on cellular processes regulated by eukaryotic homologous enzymes, which are often involved in the pathogenesis of human diseases.

1. Introduction

ADP-ribosylation (ADPr) is a reversible regulatory mechanism widespread in viruses, bacteria and in the large part of eukaryotes [1–5]. ADPr is catalysed by enzymes transferring ADP-ribose unit(s) from nicotinamide adenine dinucleotide (NAD⁺) mainly onto cellular protein substrates with the release of nicotinamide [5]. However, additional cellular macromolecules, such as DNA and antibiotics, are target of ADPr [6–8]. Throughout the evolution, ADPr has evolved in order to modulate a multitude of vital physiological processes, such as DNA damage repair [9–11], transcription [12–15], cell division [16–21], proliferation [22–25] and cell death [26–30], to cite a few. Additionally, ADPr is involved in the pathogenesis of many human diseases as well, which include neurological disorders, cancer, and

bacterial- and viral- mediated infections [4,31–36].

In the context of translational research, cellular mechanisms regulated by ADPr have attracted the attention of the scientific community, especially in the field of cancer research where the modulation of endogenous systems of ADPr has been recognised effective in sensitising cancer cells to death [37–39]. Furthermore, because of the medical need, the understanding of virulence mechanisms exploiting ADPr systems is considered equally of interest.

The cellular enzymes catalysing ADPr are named ADP-ribosyl transferases (ARTs) [40–44]. Diphtheria (DTX) and Cholera (CTX) exotoxins, respectively produced by pathogenic *Corynebacterium diphtheriae* and *Vibrio cholerae*, represent the ancestors of ART superfamily of enzymes [42,45], as well as the prototype of toxic ART exoenzymes [31,46–55]. Bacterial ARTs (bARTs) are well known causative agents of

Abbreviations: ADPr, ADP-ribosylation; NAD⁺, nicotinamide adenine dinucleotide; ARTs, ADP-ribosyl transferases; DTX, diphtheria toxin; CTX, cholera toxin; bARTs, bacterial ARTs; PAR, poly(ADP-ribose); SIRT, sirtuin; MARYlation, mono(ADP-ribosyl)ation; PARYlation, poly(ADP-ribosyl)ation; ARTDs, ADP-ribosyl transferases diphtheria toxin-like; ARTCs, ADP-ribosyl transferases cholera toxin-like; PARPs, poly(ADP-ribose) polymerases; ER, endoplasmic reticulum; ARTT, ADP-ribosylating turn-turn; A-loop, acceptor-loop; D-loop, donor-loop; MTX, mosquitocidal toxin; DarTG, DNA ART/DNA ADP-ribosyl glycohydrolase; TA, toxin-anti-toxin; PTMs, post-translational modifications; HPF1, histone PARYlation factor 1; ARHs, ADP-ribosyl-acceptor hydrolases; DraG, dinitrogenase reductase-activating glycohydrolase; PARG, poly(ADP-ribosyl) glycohydrolase; TARG1, Terminal ADP-ribose glycosylhydrolase 1; CoVs, coronaviruses; LT, heat-labile enterotoxin; PT, pertussis toxin; GPCRs, G-protein-coupled receptors; cAMP, cyclic-AMP; PKA, protein kinase A; CFTR, cystic fibrosis transmembrane conductance regulator; CDT, C. difficile toxin; CST, C. spiroforme toxin; ExoS, exoenzyme S; ExoT, exoenzyme T; RhoGAP, Rho GTPase activating protein; ERM, ezrin, radixin, and moesin proteins; PKC, protein kinase C; EF2, elongation factor-2; ChT, Cholix toxin; PDE, phosphodiesterase; Nudix, nucleoside diphosphate-linked moiety X; NPP, nucleotide pyrophosphatase/phosphodiesterase; RTN4, reticulon-4; Prs, phosphoribosyl pyrophosphate synthetase; Tre1, type VI secretion ADP-ribosyl transferase effector 1; BFA, brefeldin A; BARS, BFA-ADP-ribosylation substrate; CtBP1-S, C-terminal-binding protein-1 short-form; BAC, BFA-ADP-ribose conjugate; ARF, ADP-ribosylation factor; GEFs, guanine nucleotide exchange factors; GDP, guanosine diphosphate

* Corresponding author.

E-mail address: l.palazzo@ibp.cnr.it (L. Palazzo).

<https://doi.org/10.1016/j.bcp.2019.06.001>

Received 29 March 2019; Accepted 3 June 2019

Available online 06 June 2019

0006-2952/ © 2019 Elsevier Inc. All rights reserved.

large number of worldwide spread diseases, affecting humans, insects and plants [31,56–58], with a great impact on human health. In addition, infectious diseases strongly affect the world economy in terms of costs for the human health as well as food and agricultural economic losses [59]. The use of antibiotics to counteract the pathogen infections has represented the therapeutic strategy of choice in the last century, however the emergence of antibiotic resistance strains represents the major challenge of the 21st century [60–62]. Targeting bacterial pathogenic mechanisms by disarming pathogens of virulence factors, for instance by inhibiting the enzymatic activity of bART, represents a valid alternative intervention strategy to overcome antibiotic resistance [63–66]. In addition, because of the conservation of ADPr systems throughout the evolution, the understanding of bacterial pathogenic mechanisms may contribute to unveil novel and conserved cellular processes regulated by ADPr in high organisms [34,45,67,68]. The dysregulation of such processes can be either cause of human diseases or be target of therapeutic intervention [39,69–71].

Herein, we will provide a summary of bacterial ADPr systems describing their pathogenic mechanisms and highlighting the similarities with ADPr systems in mammals as well. Further, we discuss the potential of targeting ADPr for therapeutic strategies of infection diseases.

2. ADP-ribosylation in pathophysiology

ADPr was originally described in the 60s; two independent studies reported the identification of a new polymer, poly(ADP-ribose) (PAR) synthesised from NAD⁺ in vertebrate cells [72] and, simultaneously, the finding that bacterial DTX activity from *C. diphtheriae* is dependent on NAD⁺ content [73]. In the following decades, research in the field has expanded the involvement of ADPr in the pathophysiology of diverse cellular processes in all domains of life, which include DNA damage repair [9–11,14,74–78], cell metabolism [78–80], aging [81], cell division [16–21], transcription [12–15], cell death [26–30], bacterial and viral pathogenic mechanisms [31,33,35], defence against infection [4,24,32–34,82–84], stress response [85–89], and many others emerging pathways as intracellular trafficking [90] to refer to human cellular functions.

Because of the central role of ADPr in many essential cellular processes, the cellular signalling controlled by ADPr is finely tuned by the activity of ADP-ribosyl hydrolases. Thus, ADPr is a reversible modification. Dysregulation of ADPr signalling as well as the unbalance between transferases' and hydrolases' activities has proven to have a role in many inherited and acquired human diseases, as in several neurological disorders and in cancer [36,39,70,91–95].

3. Enzymes involved in ADPr signalling

3.1. Transferases

Two evolutionary unrelated superfamilies of enzymes catalyse ADPr; ARTs [42,45] and Sirtuins (SIRT) [96]. In this review we will not discuss about the SIRT enzymes. The majority of proteins belonging to the ART and SIRT superfamily of enzymes covalently transfer single ADP-ribose units to target proteins, thus producing mono(ADP-ribosyl)ation (MARylation) reaction [43,97]. In addition, several ARTs can transfer chains of repeating ADP-ribose units (up to 200 in length) giving rise to PAR polymers, as a result of poly(ADP-ribosyl)ation reaction (PARylation) [9,43,45,98].

3.1.1. ADP-ribosyl transferases (ARTs)

ART enzymes are widely distributed across all domains of life from bacteria to humans with exception of yeasts [2,5,31,97] and, according to the structural organisation of the ART fold, are subdivided into diphtheria toxin-like (ARTDs) and cholera toxin-like ARTs (ARTCs) classes [42,45]. Despite low sequence similarity, the two classes of ART domains share a common conserved secondary structure and protein

fold [3,42,45,54]. Diverging from the NAD⁺-binding Rossmann fold, which features oxidoreductase enzymatic activities [3], the ART protein fold consists of two central β -sheets surrounded by α -helices, with the NAD⁺ binding pocket located at the interface of the two slightly staggered β -sheets [51,99]. Three amino acids within the ART fold form a triad essential for enzymatic catalysis. The H-Y-E triad is a feature of the ARTD family, whilst the R-S-E residues characterise the ARTC group of enzymes. In detail, the histidine in position one of the ARTD's catalytic triad (H-Y-E) binds to the 2-OH of the adenosine ribose and the NH₂ of the nicotinamide amide, the tyrosine in position two π -stacks with the nicotinamide ring, and the glutamate in position three is supposed to stabilise the furanosyl oxocarbenium intermediate. Mutation of the glutamate residue in the active site of DTX decreases catalytic activity leading to loss of cytotoxicity [100–102]. In eukaryotes, ARTD enzymes are extensively known as Poly(ADP-ribose) polymerases (PARPs). The human genome encodes seventeen PARPs, which, based on variation in their catalytic triad, are divided in five groups. The first group encompasses the H-Y-E-containing enzymes (PARP1, PARP2, PARP3, PARP4, PARP5a and PARP5b). PARP1 and PARP2 catalyse synthesis of linear polymers of PAR (up to 200 units in length) through the formation of glycosidic ribose-ribose 1'-2" bonds [43,103], or of branched portions of PAR by the formation of glycosidic ribose-ribose 1"-2' linkages [104–106]. PARP5a and PARP5b (Tankyrase-1 and -2, respectively) catalyse the formation of PAR oligomers by addition of repeating units of ADP-ribose (up to 20 units in length) [9,43]. Instead, PARP3 and PARP4 catalyse MARylation [43]. Additional groups of human PARPs are the H-Y-I triad-containing enzymes (PARP6, PARP7, PARP8, PARP10, PARP11, and PARP12), the H-Y-Y-containing PARP16, the H-Y-L-containing PARP14 and PARP15, and the Q-Y-T/Y-Y-T-containing PARP9 and PARP13. With the exception of PARP13, which appears to be inactive [42,43,107], and of true poly(ADP-ribose) polymerases, the remaining human ARTD/PARP enzymes catalyse MARylation of their targets [43,108]. In addition, a divergent PARP-like enzyme containing the triad H-H-V belongs to a subgroup within the eukaryotic ARTD class [3,42] and it is referred as TpT1 or KpTA (ARTD18).

ARTCs are characterised by the R-S-E triad in the catalytic domain. Similar to the histidine within the ARTD triad, the arginine in the position one of the R-S-E triad interacts with the diphosphate backbone of NAD⁺, the serine binds to the nicotinamide ribose, and the glutamate acts as in the H-Y-E triad. ARTCs are known for their ability to modify protein substrates on arginine residues, through N-glycoside bonds (please refer to Section 3.1.3. for further details). Four ecto-ARTs (hARTC1, hARTC3, hARTC4, hARTC5) are expressed in humans, and six in mice (Artc1, Artc2.1, Artc2.2, Artc3, Artc4, and Artc5) [78,97,109,110]. Ecto-ARTs are glycosylphosphatidylinositol (GPI)-anchored proteins, with exception of Artc5 that is a secreted enzyme. Murine Artc1, Artc2 and Artc5 selectively MARylate plasma membrane-associated proteins, such as P2X7 receptor, which once modified leads to apoptotic cell death [111,112], and extracellular proteins such as the heme-binding protein hemopexin [113–116], whose ADPr reduces its capacity to bind heme. Additionally, human ARTC1 localises at the Endoplasmic Reticulum (ER) and, by modifying GRP78/BiP, functions in ER stress response [88]. Conversely, ARTC3 and ARTC4 lack the conserved residues in the catalytic triad and hence are thought to be inactive [110]. Despite the membrane-associated localisation of ecto-ARTs, several studies revealed the ADPr of arginine (Arg-ADPr) residues of soluble intracellular proteins (e.g. GAPDH, G protein $\beta\gamma$ subunits, Rabs, and tubulin) [116–119], thus suggesting that mammalian ARTCs also regulate intracellular functions.

In addition to catalytic triads, further conserved motifs are functional to the coordination of NAD⁺ or to substrate selectivity. For instance, the ARTT loop (ADP-ribosylating turn-turn), also known as the acceptor-loop (A-loop), is found in both ARTD and ARTC protein families. Studies have demonstrated the central role of the A-loop in bacterial ARTCs for targeting and selection of amino acid acceptors. The donor-loop (D-loop) is instead a common feature of ARTD enzymes

Fig. 1. Schematic representation of the ADP-ribosylation reaction onto target protein. A) Mono-ADP-ribosylation reaction is catalysed by ADP-ribosyl transferases Diptheria toxin-like (ARTDs), ADP-ribosyl transferases Cholera toxin-like (ARTCs) and Sirtuins (SIRT6) in the presence of the NAD⁺. The modification, indicated as a single red ball, is reversed by selective ADP-ribose hydrolytic enzymes (MacroD1, MacroD2, TARG1, ARH1, ARH3) that show different amino acid-ADP-ribose linkage specificity, and by phosphodiesterases (NUDT16 and ENPP1). B) Poly(ADP-ribosylation) reaction is catalysed by ADP-ribosyl transferases Diptheria toxin-like (ARTDs). Linear or branched chains of Poly(ADP-ribose), indicated as red balls, are hydrolysed by selective ADP-ribose hydrolytic enzymes (PARG, ARH3, TARG1) and by phosphodiesterases (NUDT16 and ENPP1). Further details are reported in the text. NAM, nicotinamide; ARH1/ARH3, ADP-ribosyl acceptor hydrolases 1/3; NUDT16, nudix hydroxylase 16; PARG, Poly(ADP-ribose) glycohydrolase (*endo*-glyc, *endo*-glycolytic activity; *exo*-glyc, *exo*-glycolytic activity); TARG1, Terminal ADP-ribose glycosylhydrolase 1.

that is involved in NAD⁺-binding and catalysis; conversely, it is not present in bacterial ARTCs and eukaryotic counterparts [42,45].

3.1.2. Substrates of ADPr

ARTs are mainly known in modifying proteins, however, several enzymes can modify additional molecules, such as nucleic acids (both DNA and RNA) and antibiotics [5–7,34,78]. The structure of bARTs is overall conserved in eukaryotic homologues belonging to same families, however, the selectivity for targets is clearly divergent throughout the evolution. Indeed, bARTs show a narrow target selection towards well defined protein or nucleic acid substrates. By contrast, eukaryotic ARTs show a broad ability in modifying different molecules as well as multiple residues within proteins substrates, as shown in Fig. 1 and further discussed in Section 3.1.3.

ADPr of DNA has been described for both bARTs and mammalian ARTs. Modification of DNA by ART toxins is performed by Pierisin-1 from cabbage butterfly *Pieris rapae* [120], mosquitocidal toxin (MTX) from *Bacillus sphaericus* [121], ScARP from *Streptomyces coelicolor* [122], Scabin from the pathogen *Streptomyces scabies* [123], and the non-toxin enzyme Carp-1 from shellfish *Meretrix lamarckii* [124]. Pierisin-like enzymes belong to the ARTC group and irreversibly target the N2 amino groups of 2'-deoxyguanosine in double stranded DNA *in vitro*. The cytotoxic effect of Pierisin-like enzymes has been assessed in several human cell lines, where they induce an apoptotic response [125]. Therefore, Pierisin enzymes have been suggested to function in defence mechanisms. An additional example of bART modifying DNA is provided by the reversible DarTG (DNA ART/DNA ADP-ribosyl glycohydrolase) toxin-antitoxin (TA) system encoded by *Mycobacterium tuberculosis*, *Escherichia coli* and *Thermus aquaticus* [8]. The DarT toxin, which shares homology with ARTDs, modifies endogenous DNA onto thymidine residue within a conserved single stranded nucleotide sequence. As a result of the DNA replication blockade, DarT induces a reversible bacteriostatic persistence state [8]. Further details about DarTG system are provided in Section 4.2. Mammalian DNA-damage

related PARPs are able to modify DNA as well. PARP1 and PARP2 transfer chains of ADP-ribose on phosphorylated DNA termini *in vitro* with differences; PARP1 PARylates ends of recessed DNA duplexes containing 3'-cordycepin, 5'- and 3'-phosphate and to 5'-phosphate of a single-stranded oligonucleotide; whilst PARP2 preferentially modifies nicked/gapped DNA duplexes containing 5'-phosphate at the double-stranded termini [126]. PARP3 has been shown to MARYlate phosphorylated DNA termini *in vitro* with clear preference for 5' ends of DNA double stranded breaks [7,127,128]. The biological significance of PARP-dependent ADPr of DNA in the context of DNA damage repair remains still unknown. However, reversible ADPr of DNA across various kingdoms of life implies that this modification may be more widespread than originally thought and could be responsible for modulating undiscovered cellular events, such as gene transcription and DNA replication.

Modification of RNAs by ADPr has been described for the divergent TpT1. *Saccharomyces cerevisiae* TpT1 is involved in RNA splicing by acting as tRNA 2'-phosphotransferase, catalysing the transfer of the 2'-phosphate from ligated tRNA to NAD⁺, producing mature tRNA and ADP ribose-1'-2"-cyclic phosphate at the splice junction of tRNAs [5,129–131]. Similarly, the bacterial homologue KptA, performs same reaction *in vitro* [132], though its physiological function in *E. coli* remains largely unknown since splicing is absent. Reversible ADPr of RNAs also occurs *in vitro* by the activity of the human PARP10, PARP11 and PARP15 as well as of the human homologue of TpT1/KptA TRPT1 [133]. However, the biological relevance related to PARP-mediated RNA ADPr signalling remains yet to be unveiled.

ADPr of antibiotics has been only described for Arr-ms encoded by *M. smegmatis*, which inactivates rifamycin and derivatives by modifying the hydroxyl group at C23 of the antibiotic [134,135]. According to the structural feature of the NAD⁺-binding domain, Arr-ms is a bART harbouring a divergent catalytic triad containing H-Y-D respect to the conserved H-Y-E [6]. Despite *in silico* analysis has shown that the Arr family of proteins is widely distributed in microbial communities, their

role in resistance mechanism towards antibiotics remains largely unknown.

3.1.3. Amino acids modified by ADPr

Bacterial as well as eukaryotic ARTCs are well known to MARYlate protein substrates onto basic amino acid residue arginine through N-glycosidic bonds, however other residues have been identified as targets of bARTC, such as cysteine, threonine, asparagine and glutamate [136–142]. Instead, bARTDs selectively MARYlate post-translationally modified histidine residues, termed diptamide, in protein substrates (please refer to 4.1.2 for further details). Conversely, eukaryotic ARTD/PARP group modifies a multitude of proteins targeting several amino acid residues through a selective mechanism that is still unknown. Amino acids modified by eukaryotic ARTD include serine (abbreviated as Ser-ADPr) and tyrosine (Tyr-ADPr) through O-glycosylation bonds [115,143–147], negatively charged residues such as glutamic and aspartic amino acids through ester linkages [148,149], positively charged lysine through N-glycosidic bond [150], glycine as in the case of PARP9 [108] and cysteine as reported for PARP8 [43].

More than 11,000 ADP-ribosylated peptides have been mapped in mammalian (HeLa) cells in response to DNA damage by mass spectrometric approaches [151]. Of these peptides, the fraction of Ser-ADPr accounts for 90% of the total. The 30% out of 6247 Ser-ADPr peptides harbours the lysine followed by serine motif of ADPr (KS motif) [151,152]. Strikingly, modified residues within protein substrates appear spatially restricted within cellular sub-compartments, for instance Tyr-ADPr enriches at ribosomal proteins with the most significant motif consisting of a lysine residue at the +1 position of the modified tyrosine; Arg-ADPr enriches at the ER primarily onto RNA binding proteins, with the motif flanking the arginine residue enriched with serine, and histidine-ADPr enriches at the mitochondrion [151]. Importantly, residues modified by ADPr are also target of additional post-translational modifications (PTMs), such as of phosphorylation, thus suggesting an essential role for ADPr in cross-regulating PTMs in a time-dependent fashion [144,145,147,151].

While the determinant for the high selectivity of bART for substrates is not clear (further discussed in Section 4), the ability of mammalian PARPs to modify a broad (but defined) subset of substrates could be partially dependent by protein-protein interactions, which could be responsible for substrate presentation to the enzyme. Such mechanism of substrate fishing has been demonstrated for PARP5a and PARP5b [153,154] as well as for PARP6 and PARP9 [108,155]. Same as for the specificity of PARPs in targeting well defined amino acids within protein substrates. A well-studied example of specificity for modification sites within protein substrates induced by interacting proteins has been shown for PARP1. The interaction of PARP1 with histone PARYlation factor 1 (HPF1) induces PARP1-dependent *in trans* modification of histones and other DNA repair factors onto serine residues, thus limiting the ability of PARP1 to automodify onto acidic residues [76,144,152].

3.2. ADP-ribosyl hydrolases

ADPr is dynamically regulated by the activity of specialised ADP-ribosyl hydrolases, which reverse the modification once the cellular response has been achieved (Fig. 1) [5,156,157]. Two evolutionary unrelated protein families are known to support this function: DraG-like ADP-ribosyl-acceptor hydrolases (ARHs) and macrodomain-containing enzymes.

DraG-like fold-containing class consists of enzymes which shares homology with the founder dinitrogenase reductase-activating glycohydrolase (DraG), firstly characterised in bacteria [158–167]. DraG is a Mg^{2+} -dependent arginine-hydrolase known to counteract the activity of the ART endotoxin DraT in the photosynthetic bacterium *Rhodospirillum rubrum* [168] (detailed in Section 4.2). The human genome encodes three DraG-related ARHs (ARH1, ARH2, and ARH3), which

play roles as regulators of cellular stresses [157,161,163,164]. Similarly to bacterial DraGs, ARH1 reverses arginine ADPr operated by mammalian ARTCs as well as bacterial toxins [162,167]. In this regards, ARH1-deficient mice, exhibited enhanced sensitivity to Cholera toxin [169,170], thus suggesting that modification of cellular substrates performed by bARTs can be reverted by mammalian hydrolases [162]. ARH3 shows activity in hydrolysing O-glycosidic bond, and it is highly effective in reversing Ser-ADPr-ribosylated substrates [164], while ARH2 appears to be inactive.

Macrodomain-containing proteins share a common ADP-ribose binding domain, known as macrodomain. Macrodomains are widely distributed in all kingdoms of life from viruses to humans, suggesting their evolutionary conservation [156,171] and play crucial roles in the recognition and hydrolysis of ADPr in different cellular contexts [172]. Macrodomain-containing proteins coupled to mass spectrometry analysis have represented so far a powerful tool for *in vitro* identification of MARYlated or PARYlated substrates [75,118,145,151,173–175]. In addition to the ability of interacting with ADP-ribose or PAR or O-acetyl-ADP-ribose, several macrodomains have acquired ADP-ribosyl hydrolase activity, showing distinct substrate specificity. Macrodomain-containing hydrolases include the poly(ADP-ribosyl) glycohydrolase (PARG), MacroD1, MacroD2 and Terminal ADP-ribose glycosylhydrolase 1 (TARG1) [156,171,172,176–178]. PARG is the only macrodomain-containing enzyme that efficiently cleaves PAR chains through exo- or endo-glycosyl hydrolytic activity, though it is unable to remove the terminal ADP-ribose linked to protein substrates [176]. Conversely, MacroD1, MacroD2, as well as TARG1 specifically revert MARYlation by hydrolysing the bond between the terminal ADP-ribose and amino acid side chain residues on acidic residues [92,156,179,180]. Macrodomain-containing hydrolases have been also found in bacterial genomes, though their functional role in bacterial metabolism is poorly investigated [181,182]. Bacterial macrodomain-containing hydrolases prevalently revert MARYlation. However, some macrodomains, such as the TARG1-type macrodomain enzyme from *Fusobacterium mortiferum* ATCC 9817 [183] and the bacterial PARG from *Thermomonospora curvata* [176], have been described for the capability of hydrolysing chains of ADP-ribose, which can be produced by bacterial PARPs, such as by the *Herpetosiphon aurantiacus* PARP [176]. In addition, an endogenous bacterial PARG processing enzyme activity has been characterised in *Deinococcus radiodurans* to counteract the huge amounts of PAR accumulated after UV irradiation [184]. The identification of enzymes able to produce and degrade polymers of ADP-ribose suggests that, similarly to human counterparts, PARYlation occurs in certain bacteria and it may have roles in response to genotoxic stresses.

In addition, macrodomain-containing hydrolases are encoded by viral genomes. By reversing defensive host ADPr mechanisms, viral macrodomains have been reported to support replication of viruses and their pathogenic mechanisms, thus leading to evasion of the host immune response [33,35,185]. Of note, macrodomain-containing hydrolases encoded by members of *Coronaviruses* (CoVs) have been also reported to modulate the production of interferon by erasing the ADPr-dependent signalling of the host that is required for interferon induction [186]. For further details about viral macrodomains, we refer the reader to valuable and specialised reviews in the field [33,35,185].

4. ADPr reaction in bacteria

Bacterial ARTs are involved in the regulation of bacterial metabolism and growth as well as in infection processes [56,187]. While the endotoxins act modifying bacterial effectors allowing bacteria to survive under unfavourable environmental conditions, the exotoxins are instead virulence factors that, by transferring ADP-ribose onto host targets, contribute to bacterial pathogenesis (Table 1).

Table 1
Bacterial exo- and endo-toxins with ADP-ribosyl transferase activity.

Exotoxins					
Bacterium	Toxin	Target	Effect	Role in pathogenesis	Reference
CTX-like Toxin modifying G proteins					
<i>Vibrio cholerae</i>	CTX	Gas (Arg201)	Gas activation	cAMP induction	[192]
<i>E. coli (ETEC)</i>	ET	Gas (Arg201)	Gas activation	cAMP induction	[193]
<i>Bordetella pertussis</i>	PT	Gai (Cys351)	Gai inactivation	cAMP induction	[195]
CTX-like Toxins modulating actin cytoskeleton and intracellular trafficking					
C2-like					
<i>Clostridium botulinum</i>	C2	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[211]
<i>Clostridium perfringens</i>	Iota	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[212]
<i>Clostridium difficile</i>	CDT	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[213]
<i>Clostridium spiroforme</i>	CST	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[213]
<i>Bacillus cereus</i>	VIP	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[213]
<i>Salmonella</i> sp.	SpvB	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[214]
<i>Photorhabdus luminescens</i>	Photox	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[137]
<i>Aeromonas hydrophila</i>	VgrG1	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[215]
<i>Neisseria meningitidis</i>	Nar E	Actin	Actin Depolymerisation	Alteration of cytoskeleton	[200]
<i>Aeromonas salmonicida</i>	AexT	Actin (Arg177)	Actin Depolymerisation	Alteration of cytoskeleton	[216]
<i>Photorhabdus luminescens</i>	Tc (TccC3 Subunit)	Actin (Thr148)	Actin clustering	Alteration of cytoskeleton	[137]
C3-like					
<i>Clostridium botulinum</i>	C3bot	Rho A/B/C (Asn41)	Rho protein inactivation	Alteration of cytoskeleton	[210]
<i>Staphylococcus aureus</i>	C3Stau (EDIN)	Rho A/B/C (Asn41)	Rho protein inactivation	Alteration of cytoskeleton	[210]
<i>Bacillus cereus</i>	C3cer	Rho A/B/C (Asn41)	Rho protein inactivation	Alteration of cytoskeleton	[210]
<i>Clostridium limosum</i>	C3-lim	Rho A/B/C (Asn41)	Rho protein inactivation	Alteration of cytoskeleton	[210]
<i>Photorhabdus luminescens</i>	Tc (TccC5 subunit)	Rho A/B/C (Gln61 or 63)	Rho protein activation	Alteration of cytoskeleton	[137]
<i>Paenibacillus larvae</i>	Plx2	Rho A	Rho A protein inactivation	Reorganisation of actin cytoskeleton	[140]
<i>Pseudomonas aeruginosa</i>	ExoS	Rab7, Rab8, Rab11 (Arg) Rab5 (Arg 81, 91, 110, 120) Ras (Arg 41, 123) ERM proteins (Moesin - Arg 553, 560, 563)	Inhibition of Rab function Ras inactivation Rho protein inactivation	Inhibition of endocytosis Uncoupling Ras signal transduction Cytoskeleton alteration	[230] [234] [234]
<i>Pseudomonas aeruginosa</i>	Exo T	CRK I/II (Arg)	Uncoupling integrin signalling	Cytoskeleton alteration	[235]
<i>Enteropathogenic E. coli (EPEC)</i>	EspJ	Src (Glu310)	Src inactivation	Inhibition of phagocytosis	[141]
<i>Salmonella enterica salamae</i>	SeoC	Src (Glu310)	Src inactivation	Inhibition of phagocytosis	[142]
<i>Salmonella bongori</i>	SboC	Src (Glu310)	Src inactivation	Inhibition of phagocytosis	[142]
DTX-like Toxins modulating machinery of protein synthesis					
<i>Corynebacterium diphtheriae</i>	DTX	EF2 (Diphthamide715)	Inhibition of EF2 function	Inhibition of translation	[53]
<i>Pseudomonas aeruginosa</i>	Exo A	EF2 (Diphthamide715)	Inhibition of EF2 function	Inhibition of translation	[237]
<i>Vibrio cholerae</i>	ChT	EF2 (Diphthamide715)	Inhibition of EF2 function	Inhibition of translation	[238]
Toxins altering the ubiquitin signalling					
<i>Legionella pneumophila</i>	SdeA	RTN4, Rab33b, Rab1, Rab6a and Rab30 (Ser)	Misregulation of Rab and RTN4 functions	Alteration of intracellular traffic	[244]
Endotoxins					
Bacterium	Toxin	Target	Effect	Function	Reference
<i>Rhodospirillum rubrum</i>	DraT	Nitrogenase reductase (Arg101)	Inhibition of enzymatic activity	Deregulation of nitrogen fixation system	[168]
<i>Azospirillum brasilense</i>	DraT	Nitrogenase reductase (Arg101)	Inhibition of enzymatic activity	Deregulation of nitrogen fixation system	[168]
<i>Mycobacterium tuberculosis</i>	DarT	Single-stranded DNA (Thymidine)	Possibly altering DNA transcription/replication	Down regulation of cell metabolism	[263]
<i>Sphingobium</i> sp	ParT	Phosphoribosyl pyrophosphate synthetase (Prs)	Inhibition of enzymatic activity	Altering nucleotide biosynthesis	[267]
<i>Serratia proteamaculans</i>	Tre1	FtsZ	Alteration of bacterial cytoskeleton	Bacteria cell death	[268]

4.1. ADPr as a mechanism of pathogenesis

Bacterial exotoxins exert their pathogenic role through MARYlation of proteins within the host mainly involved in signal transduction, organisation of cellular cytoskeleton and membrane trafficking, and protein synthesis (Fig. 2) [188]. However, many other unknown cellular substrates in the host may be target of bART, thereby further mass spectrometric studies are required to expand the understanding of mechanisms of action operated by bART.

The bART family includes more than 35 toxin members that differ

for several aspects, such as substrate specificity, host (plants, insects or human), mechanism of action, as well as protein domain organisation [31]. As such, in the majority of cases, exotoxins are composed by two domains referred as A:B. The A domain corresponds to catalytic ART domain, whilst the B domain corresponds to the “binding” portion that is responsible for the binding to specific cell receptors [31,188]. Domains are differently arranged in bART toxins, for instance DTX is a single A-B polypeptide chain, CTX is a A5B toxin, C2-like toxins are binary toxins, due to the presence of the A and B polypeptide chains independently expressed, C3-like toxins are single A polypeptide

Fig. 2. Schematic representation of eukaryotic cellular mechanisms modulated by virulence factors displaying ADP-ribosylation activity.

chains, as previously reviewed [31].

Thus, despite molecular and biochemical divergences, bARTs can be roughly classified into two classes, based on sequence similarities of the catalytic ART fold domain; namely the CTX-like and DTX-like [31], as described in section 3.1.1. Accordingly, we discuss the bARTs grouping them into the two main classes and then describing the substrate specificity and the pathogenic mechanisms of representative toxins (summarised in Table 1). In addition, we report with details divergent and remarkable mechanisms of non-canonical ADPr of some toxins.

4.1.1. Cholera Toxin-like

bARTCs are currently subdivided in three sub-groups reflecting the specificity of toxins for intracellular targets, namely CTX-like toxins that modify small GTPases involved in signal transduction, C2-like toxins that modify actin, and C3-like toxins that interfere with membrane trafficking of the host. In addition, other non-canonical bARTC have been identified for their ability to modify cellular kinases.

The CTX-like group of toxins includes the well-known CTX from *V. cholerae*, heat-labile enterotoxin (LT) from *E. coli* and pertussis toxin (PT) from *Bordetella pertussis*. CTX and PT modify G-proteins, also known as guanine nucleotide-binding proteins. G-proteins are heterotrimeric molecules composed by alpha (α), beta (β) and gamma (γ) subunits, and are required to transmit the information from G-protein-coupled receptors (GPCRs) on the plasma membrane to the inside of cells. Depending on the targeted cell types, signals transduced by GPCRs modulate diverse physiological functions, such as the gating of polarisation of cardiac cells, the smooth muscle tone, the carbohydrate and lipid metabolism, immune cell effector functions [189]. CTX and LT bind GM1 ganglioside receptor on the cell surface and then are internalised through the endocytic pathway. In the cytoplasm, the toxin specifically modifies the Gas at arginine residue 201 [190,191]. The ADP-ribosylated Gas subunit becomes locked in a GTP-bound state resulting in a constitutive activation of host adenylate cyclase, which produces cyclic-AMP (cAMP). Consequent elevated intracellular levels of cAMP induce activation of protein kinase A (PKA), which in turn phosphorylates and activates the cystic fibrosis transmembrane conductance regulator (CFTR) protein, causing an efflux of ions (sodium and potassium) and water from infected enterocytes into the intestinal lumen, thus leading to severe diarrhea [192]. Similarly to CTX mechanism of action, LT deregulates the ion transport efflux on the luminal surfaces of intestinal epithelial cells, thus favouring intestinal water secretion into the lumen of the bowel and secretory diarrhea

[193,194]. Conversely, PT ADP-ribosylates the inhibitory $G_{i\alpha}$ at cysteine 351 residue, which locks the $G_{i\alpha}$ in GDP-bound inactive state, thus leading to a constitutive active cAMP signalling [195–197]. Similar cytotoxic effects have been also described for additional PT-like toxins recently characterised from several *Salmonella* species such as *S. typhimurium* (ArtAB-DT104), *S. worthington* (ArtAB-SW) and *S. bongori* (ArtAB-Sb) [139,198].

A divergent CTX-like is represented by NarE toxin, which is produced by *Neisseria meningitidis*. This gram-negative bacterium is responsible for meningitis and fulminant septicemia in humans [199]. Even though NarE toxin shares structural homologies with LT and CTX, it shows different cellular targets. Notably, NarE is a dual enzyme with both ART and NAD^+ -glycohydrolase activities. The ART activity of NarE is induced by the presence of target acceptor proteins, such as different cytoskeleton-related proteins, including actin [200]. By contrast, NarE displays a NAD^+ glycohydrolase activity in the absence of substrates [201,202]. At the cellular level, NarE intoxication causes the disruption of epithelial integrity of upper respiratory tract and the induction of apoptotic pathway leading to cell death [200].

bART belonging to C2-like and C3-like toxins converge their activities in modulating, directly or indirectly, the homeostasis of intracellular trafficking processes within the host. The intracellular vesicular transport allows proteins and enzymes to be sorted at specific subcellular compartments through the formation of vesicular intermediates. By doing so, membrane traffic contributes to maintain cell homeostasis [203]. The formation, targeting and fusion of vesicles that transport cargos among different compartments rely on the activity of small GTPases, such as members of the Ras superfamily of monomeric G proteins (Rab) [204,205], the ADP-ribosylating factors (ARFs) [206] and members of the Rho family of GTPases [207]. Intracellular vesicles are transported along cytoskeletal components such as actin filaments or microtubules, which serve a structural role in cell shape and architecture [208,209]. Thereby, MArlylation of cellular components of the intracellular transport by bARTs impairs host intracellular trafficking processes, thus sustaining the infection process [210]. The C2-like group includes many toxins from different bacteria among which C2 toxin from *Clostridium botulinum*, Iota toxin from *C. perfringens*, *C. difficile* toxin (CDT) from *C. difficile*, *C. spiroforme* toxin (CST) from *Clostridium spiroforme*; VIP2 toxin from *B. cereus*, SpvB from *Salmonella* sp., as well as VgrG1 and AexT from *Aeromonas hydrophila* and *Aeromonas salmonicida*, respectively [211–216]. C2-like exotoxins usually modify actin at Arg177. ADPr of actin leads to the disassembly of actin

filaments and, in turn, impairs cellular motility, migration as well as deregulation of intracellular trafficking [210,217]. For instance, CDT-induced ADPr of actin results in erroneous delivery of intracellular vesicles from the basolateral to the apical side of epithelial cells where extracellular matrix proteins, such as fibronectin, are released, thus promoting host-pathogen interaction [218].

Actin is also a target of TccC3 subunit, a component of the tripartite Tc toxins from *Photobacterium luminescens*, an emerging gram-negative human pathogen. TccC3 modifies actin at threonine 148, which, contrary to C2-like toxins, induces actin polymerisation and clustering [137].

Members of the C3-like group encompass exoenzymes from *C. botulinum*, *Staphylococcus aureus*, *B. cereus*, *C. limosum* and *Paenibacillus larvae*. C3-like exoenzymes selectively modify the small GTP-binding proteins RhoA/B/C at a conserved Asn residue 41 [140,219–221]. RhoA/B/C GTPases are small-molecular weight G-proteins, which modulate the state of actin cytoskeleton polymerisation. Pathogen-mediated ADPr renders Rho biologically inactive and leads to a lethal redistribution of actin in the target cell [222–224]. Cellular consequences are the alteration of epithelial and endothelial barrier functions [225,226], the impairment of the immune cell signalling including phagocytosis [227]. Rho GTPases are also targeted by TccC5 subunit of Tc toxin from *P. luminescens* [137], though onto different residues (Gln61 or 63). Exoenzyme S and T (ExoS and ExoT) from *Pseudomonas aeruginosa*, which are additional C3-like toxins, are characterised by the presence of a Rho GTPase activating protein (RhoGAP) domain in their sequence [228,229]. ExoS modifies several Rab GTPases, e.g. Rab5, Rab7, Rab8, Rab11, thus impairing normal endocytic pathways. For instance, ADPr of Rab5 onto multiple arginine residues (Arg81, 91, 110, and 120) prevents tethering and fusion of endocytic vesicles with early endosomes [230]. As a consequence, plasma membrane-associated receptors, such as epithelial growth factor receptor (EGFR), are trapped in a clathrin-coated vesicle [56]. ExoS also modifies ezrin, radixin, and moesin (ERM) proteins, which are regulators of the actin network through the activation of Rho protein [231]. ADPr of ERM proteins on arginine residues of moesin (Arg553, Arg560 and Arg563) impairs the phosphorylation of ERM protein components operated by the protein kinase C (PKC) and Rho kinases, thus leading to actin cytoskeleton and trafficking alterations. Modulation of ERM functions by ADPr ultimately leads to actin cytoskeleton and trafficking alterations [232,233]. Furthermore, ExoS ADP-ribosylates Ras GTPase at arginine residues (Arg41 and 123) resulting in its inactivation and thus inhibition of Ras signal transduction, which is responsible for cell growth and survival [234].

ExoT recognises two cytosolic proteins as substrates, Crk-I and Crk-II respectively. Both proteins are Src homology 2–3 domains containing adaptor proteins involved in focal adhesion and phagocytosis. ExoT-mediated ADPr of Crk I/II impairs the interaction between Crks and focal adhesion proteins, causing alteration of integrin signalling pathway, which ultimately leads to actin cytoskeleton rearrangement [235,236].

Further, bARTC effectors have been described to modify cellular kinases. The *E. coli* effector EspJ, as well as the SeoC and SboC homologues from *Salmonella enterica* subsp. *salamae* and *arizonae* and *S. bongori*, modifies the highly conserved glutamic residue at position 310 (Glu310) on Src protein to inhibit phagocytosis. In addition, EspJ modifies by ADPr other non-receptor tyrosine kinases, such as the Abl, Csk, Tec, and Syk, leading to inhibition of both autophosphorylation and phosphorylation protein substrates [141,142].

4.1.2. Diphtheria toxin-like

DTX is secreted by *C. diphtheriae* and targets the eukaryotic elongation factor-2 (EF2) as substrate. DTX-dependent ADPr occurs at specific histidine residue (His715); the modified histidine is termed diphthamide. Diphthamide is located in the region of EF2 responsible for the interaction with components of translation machinery, thus

ADPr of EF2 acts as an inhibitor of RNA translation and protein synthesis [46,53].

DTX infection leads to necrosis of the heart and liver. The exotoxin A (ExoA) secreted by *P. aeruginosa* as well as the Cholix toxin (ChT) secreted by *V. cholerae* share structural and functional similarities with DTX and both of them modify EF2 with similar functional consequences [237–239].

4.1.3. ADP-ribosylation-dependent ubiquitination as mechanism of pathogenesis

Legionella pneumophila is an opportunistic pathogen that typically spreads via inhaled aerosols and is the causative agent of the so-called Legionnaires' disease, inducing pneumonia. *L. pneumophila* manipulates host functions to achieve the formation of the Legionella-containing vacuole (LCV), a replicative niche necessary for bacterial growth and survival [240–242]. One common strategy adopted by pathogens is the inactivation of ubiquitin-dependent inflammatory signalling pathways [243]. Despite *Legionella* lacks ubiquitination system, it contains a multitude of proteins able to interfere with host ubiquitination machinery. The SidE protein family, which encloses SidE, SdeA, SdeB, and SdeC proteins, contributes to *Legionella* pathogenesis [244,245]. All SidE proteins contain an ARTC-like motif required for their toxicity to yeast [246]. When expressed in mammalian cells, SidE family effectors were found to induce an E1 and E2-independent ubiquitination of several ER-associated Rabs, including Rab33b, Rab1, Rab6a and Rab30 [244]. By clarifying the virulence mechanism of SdeA, it has been shown that the ubiquitination induced by *Legionella* in the host is dependent on ADPr [245]. Structural and biochemical characterisation have revealed that SdeA is a dual enzyme with both MARYlation and phosphodiesterase (PDE) activities, due to the presence of two distinct protein folds, namely ARTC-like and PDE domains [245,247,248]. ADPr-dependent ubiquitination of protein substrates catalysed by SdeA is a two-step reaction: first, SdeA transfers ADP-ribose from NAD⁺ onto arginine 42 (Arg42) of an Ubiquitin (Ub) molecule in order to generate an ADP-ribosylated-Ub intermediate; in the second step, phosphodiesterase activity converts ADP-ribosylated-Ub intermediate to phosphoribosyl-Ub which is then conjugated through an ester linkage to a serine residue to target protein or to SdeA itself [245,248]. Thus, phosphodiesterase activity of PDE is strictly required to generate a phosphoribosyl-Ub intermediate. The enzymatic step of hydrolysis of the phosphodiester bond within ADP-ribosylated Ub by SdeA resembles the activity displayed by some enzymes belonging to the nucleoside diphosphate-linked moiety X (Nudix) and nucleotide pyrophosphatase/phosphodiesterases (NPP). Enzymes performing such hydrolytic reaction enclose NUDT16 and the bacterial EcRppH as well as ENPP1 and the reptile Phosphodiesterase 1 [249–251].

As a result of SdeA-mediated phosphoribosyl ubiquitination of protein targets, i.e. Rab33b, Rab1, Rab6a, Rab30 [244], and ER-associated protein Reticulon-4 (RTN4), the physiological system of ubiquitination cascade is impaired, thus affecting physiological mechanisms requiring this modification, such as mitophagy (Fig. 3) [245,252]. SdeA catalytic mechanism is inhibited by adenosine 5'-O-thiomonophosphate, an AMP analogue, that binds with low-affinity the SdeA PDE domain and alters substrate positioning. This finding provides new insights for a rationale designing of novel antimicrobial compounds to inhibit this class of *Legionella* toxins [248].

SidJ encoded by *L. pneumophila* counteracts SidE toxicity [246,253]. As prolonged activation of SdeA might be also unfavourable for bacterial growth inducing host cell death [254], *Legionella* may have evolved SidJ as a deubiquitinase enzyme described for its ability to hydrolyse the phosphodiester linkage of phosphoribosyl-ubiquitinated substrates [255].

4.2. Endogenous bacterial ADPr and cell homeostasis regulation

Cell homeostasis is ensured by the activity of metabolic enzymes,

Fig. 3. Schematic representation of *Legionella pneumophila* infection mediated by SdeA effector. By transferring a phosphoribosyl group on ER-resident substrates, such Reticulon-4 (RTN4) and several Rab proteins, SdeA induces the formation of ER-derived vesicles and their association with the Legionella-containing vacuoles (LCVs). LCVs also make contacts with ER and mitochondria, a phenomenon that affects physiological processes, such as ER-phagy and mitophagy.

regulated according to environmental conditions. As such, nitrogenase reductase enzymatic activity, responsible for nitrogen fixation (i.e. the conversion of nitrogen to ammonium), is modulated through endogenous MARYlation in the photosynthetic nitrogen-fixing bacteria *Rhodospirillum rubrum* and *Azospirillum brasilense* [168,256,257]. This mechanism has been extensively studied in the alphaproteobacteria and bioinformatics analysis supports the idea that a similar mechanism is active in at least 25 bacterial genera [257,258]. The reaction is catalysed by the dinitrogenase reductase ADP-ribosyl transferase (DraT). DraT shows a very low sequence similarity with bacterial ARTD and ARTC members, suggesting the idea that new enzymes may contribute to expand the role of ADPr.

DraT modifies the dinitrogenase reductase at arginine 101, which leads to enzymatic activity loss and shutting-off the nitrogen fixation. The reversal of DraT-dependent ADPr is performed by a specific ADP-ribosylarginine glycohydrolase (DraG), thus fully restoring dinitrogenase reductase activity [168]. The DraT and DraG activities are subjected to opposite regulation *in vivo*. In response to negative stimuli, such as exposure to darkness or high concentrations of ammonium, DraT promotes the inactivation of nitrogenase by ADPr. Conversely, when the negative stimulus is removed, namely light illumination or exhaustion of ammonium in turn, the enzymatic activity of DraG is stimulated, thus restoring nitrogenase function [259].

Mechanisms of ADPr are also part of toxin-antitoxin (TA) systems, potent tools exploited by bacteria in order to modulate fundamental bacterial processes in response to environmental stimuli. TA systems are indeed required to induce dormancy and persistence in bacteria under hostile environmental conditions and to form biofilms as well [260–262]. TA systems are also involved in the pathogenesis of several infectious diseases caused by *M. tuberculosis* [263], *E. coli* [264], *Haemophilus influenzae* [265], and *S. typhimurium* [266]. Toxin-antitoxin (TA) modules consist of a stable toxin and of a cognate anti-toxin, mostly encoded in an operon, whose co-expression is co-regulated at transcriptional and translational level. An ADPr-dependent TA system is encoded by the pathogen *M. tuberculosis*. The toxin DarT MARYlates the

second thymidine base within the TNTC conserved sequence of single-stranded chromosomal bacterial DNA. As a result of this modification, DarT interferes with DNA replication or transcription, consequently leading to bacterial growth arrest and dormancy. DNA modification is reversed by the cognate anti-toxin macrodomain-enzyme (DarG). As DarTG system induces bacteriostatic effects, it represents a promising candidate in drug design for developing novel anti-virulence strategies [8].

Additional TA systems involving ADPr encompasses ParST encoded by the bacterium *Sphingobium* sp. YBL2 [267]. ParT exerts bacteriostatic effects via ADPr of phosphoribosyl pyrophosphate synthetase (Prs), an essential enzyme in nucleotide biosynthesis conserved in all organisms. The bacteriostatic state is reversed by interaction with the cognate anti-toxin ParS, which does not act as a hydrolase enzyme.

Further, Tre1 (type VI secretion ADP-ribosyl transferase effector 1) from *Serratia proteamaculans* is a novel bARTC toxin with a role in inter-bacterial species competition; it has been recently reported to modify the critical microbial cell division protein FtsZ, the tubulin-like protein. Following the modification, FtsZ loses its ability to polymerise resulting in the alteration of cytoskeletal structure and leading to cell death. The ARH-like hydrolase Tri1 can revert the modification [268].

5. The BFA-mediated ADP-ribosylation-like reaction

Brefeldin A (BFA) has been described as a non-canonical ADPr toxin [269]. Although BFA is a non-bacterial toxin, we discuss it because of its peculiar mechanism of ADPr. BFA was indeed isolated as macrocyclic lactone from fungal organisms such as *Eupenicillium brefeldianum* and *Alternaria carthami*, where it is synthesised starting from palmitate [270]. BFA has been shown to induce ADPr of two eukaryotic proteins; GAPDH and a protein of 50 kDa [269], which was named BARS (BFA-ADP-ribosylation substrate). BARS was later identified as a member of C-terminal-binding protein-1 short-form (CtBP1-S/BARS), member of the CtBP family [271]. CtBP1-S/BARS is a protein involved in two specific functions, one in the cytoplasm and the other in the nucleus [272]. In the cytoplasm, CtBP1-S/BARS is involved in membrane fission that controls the formation of post-Golgi carriers [273–275] endocytic fluid-phase carriers [276], and COP1-coated vesicles [277], and it is also involved in mitotic Golgi partitioning [278]. In the nucleus, members of the CtBP protein family act as transcription co-repressors, and thus regulate numerous cellular functions, including epithelial differentiation, tumourigenesis and apoptosis [279]. The BFA-dependent ADPr of CtBP1-S/BARS is a non-classical two-step reaction [280]. The first step is a novel form of ADPr of a small molecule resulting from the covalent binding of ADP-ribose derived from NAD⁺ or cyclic ADP-ribose to BFA. This reaction leads to the formation of a BFA-ADP-ribose conjugate (BAC). ADP-ribosyl cyclases, such as CD38, are the enzymes responsible for the synthesis of BAC, which happens in the extracellular space. In a second step, BAC efficiently internalises through the CD38 channel-like dimeric structure and conjugates with NAD(H)-binding Rossmann fold of CtBP1-S/BARS [280,281]. According to the model, the C3 atom of BFA falls in close proximity with the imidazole ring of His304 of CtBP1-S/BARS [280]. Notably, the proposed mechanism for BAC binding to CtBP1-S/BARS, which relies on the His304/Glu289/Arg86 (H-E-R) triad, is in agreement with the structural similarities between CtBP1-S/BARS and D2-hydroxy acid dehydrogenases [281], where the structurally equivalent H-E/D-R triad is involved in substrate binding and dehydrogenase activity. Of note, BAC selectively binds the Rossmann fold of BARS, but not that of other dehydrogenases [280]. From the functional point of view, the new BFA-dependent ADPr reaction affects the cytoplasmic fission-inducing activity of CtBP1-S/BARS. BAC impairs the binding of CtBP1-S/BARS to partners involved in fission activity, thereby inhibiting BARS-dependent mitotic Golgi fragmentation. This event results in a potent and prolonged cell-cycle block in G2 phase of the cell cycle [280].

Notably, in addition to the cytotoxic effects mediated by ADPr, BFA

also acts as a non-competitive inhibitor of the ARF guanine nucleotide exchange factors (GEFs). BFA binds the transient ternary complex that is formed by ARF, GDP and the GEF Sec7 [282] inducing the release of a set of proteins from the Golgi complex [283]. Based on the latter property, BFA has been used as a research tool to analyse the mechanisms of intracellular membrane transport. Indeed, the main effects of BFA are a dramatic morphological reorganisation of the Golgi complex and redistribution of both resident and cargo proteins from the Golgi membranes to the ER [284–286]. BFA also affects the morphology and function of the endosomal/lysosomal compartments [287]. BFA has shown several biological and potent activities such as antiviral [288], antifungal [289], antitumoural and apoptosis-inducing properties in several cancer cell lines [290–292].

6. Conclusions: targeting toxin ADP-ribosyl transferase activity

Antibiotics have been the treatment of choice for bacterial diseases for long time, however, because of the growing bacterial resistance to antibiotics, alternative solutions have been searched to help limit the severity of the infection [64]. Novel antimicrobial approaches rely on targeting specific bacterial toxins causative of virulence instead of impairing bacterial processes common with non-pathogenic resident microbiota. In the last decade, strategies raised against toxins have been developed according to their diverse mechanism of action, for instance by inhibiting enzymatic activities of toxins [65,293]. Targeting of toxin ART activity mainly relies on chemical modulation of NAD⁺-interaction pocket inside the ART domain to block enzymatic activity. Initial approaches have foreseen the use of PJ34, a non-specific inhibitor, largely known for targeting human PARPs. Although PJ34 has been shown to inhibit efficiently *P. aeruginosa* exotoxin-A ($K_i = 140$ nM), it was also able to target endogenous PARPs in the mouse models of stroke, therefore lacking the specificity to ART toxins, needed for therapeutic administration. In order to address the specificity of inhibitors for bART, drug design approach studies have been performed by using the co-crystal structure of DXT or ChT in complex with PJ34 as template for virtual screening [294]. 500,000 commercially available molecules and a small library of twelve known PARP inhibitors have been analysed. Such approach led to the identification of a set of lead compounds capable of inhibiting ChT and ExoA, and CTX exotoxin ART activity both *in vitro* and in cell-based assays [294]. Further, additional strategies have instead exploited the antimicrobial protection provided by polyphenolic compounds from grape extract [295]; the screening of individual compounds has led to identify twelve molecules active against CTX, where four out of twelve acting through inhibition of ART activity. Similarly, molecules inhibiting ExoS ART activity with an IC_{50} of 1.3 μ M have been proposed as starting point for a precise targeting of virulence factors [296]. The high conservation of ADPr mechanisms throughout the evolution suggests that in-depth studies are needed in order to ensure that therapeutic molecules targeting bacterial toxins would not be specific for endogenous mechanisms of ADPr in the host, whose alteration may cause serious side effects.

Exploitation of bacterial Toxin-Antitoxin systems relying on ADPr mechanism could represent an innovative strategy to fulfil the requirement of new, non-canonical targets. TA systems have no human counterparts, are widespread in bacterial genomes, thus representing alternative targets for development of antimicrobial compounds. Of note, inhibition of the anti-toxin component (such as DarG), either by the administration of selective inhibitors or by silencing (i.e. by sequence-specific antisense agents), could be envisaged as a novel therapeutic strategy [297].

However, despite the great efforts in developing anti-virulence compounds or in applying available PARP inhibitors for targeting ART activities involved in infectious diseases, the use of blocking antibodies still represents the gold standard treatment for neutralising bacterial toxins [63,64,298–300].

Declaration of Competing Interest

The authors declare no conflict or financial interests.

Acknowledgements

The authors wish to thank Dr Ivan Ahel (Sir William Dunn School of Pathology, University of Oxford) and Dr Daniela Corda (Institute of Protein Biochemistry, National Research Council of Italy) for helpful comments on the manuscript. L.P. received fellowship from the Italian Foundation for Cancer Research [FIRC, Milan, Italy; ID:14895]. The authors' work was supported in part by PRONAT project, the SATIN POR project 2014-2020 and the Italian MIUR Cluster project Medintech (CNT01_00177_962865). C.V. acknowledges TRansforming IDEas in Oncological Research grant from the Italian Association for Cancer Research [TRIDEO, AIRC-Fondazione Cariplo, Milan, Italy; grant IG17524].

References

- [1] M. Citarelli, S. Teotia, R.S. Lamb, Evolutionary history of the poly(ADP-ribose) polymerase gene family in eukaryotes, *BMC Evol. Biol.* 10 (2010) 308.
- [2] D. Perina, A. Mikoc, J. Ahel, H. Cetkovic, R. Zaja, I. Ahel, Distribution of protein poly(ADP-ribosylation) systems across all domains of life, *DNA Repair (Amst)* 23 (2014) 4–16.
- [3] L. Aravind, D. Zhang, R.F. de Souza, S. Anand, L.M. Iyer, The natural history of ADP-ribosyltransferases and the ADP-ribosylation system, *Curr. Top. Microbiol. Immunol.* 384 (2015) 3–32.
- [4] M.D. Daugherty, J.M. Young, J.A. Kerns, H.S. Malik, Rapid evolution of PARP genes suggests a broad role for ADP-ribosylation in host-virus conflicts, *PLoS Genet.* 10 (5) (2014) e1004403.
- [5] L. Palazzo, A. Mikoc, I. Ahel, ADP-ribosylation: new facets of an ancient modification, *FEBS J.* 284 (18) (2017) 2932–2946.
- [6] J. Baysarowich, K. Koteva, D.W. Hughes, L. Ejim, E. Griffiths, K. Zhang, M. Junop, G.D. Wright, Rifamycin antibiotic resistance by ADP-ribosylation: Structure and diversity of Arr, *Proc. Natl. Acad. Sci. U.S.A.* 105 (12) (2008) 4886–4891.
- [7] D. Munnur, I. Ahel, Reversible mono-ADP-ribosylation of DNA breaks, *FEBS J.* 284 (23) (2017) 4002–4016.
- [8] G. Jankevicius, A. Ariza, M. Ahel, I. Ahel, The toxin-antitoxin system DarTG catalyzes reversible ADP-ribosylation of DNA, *Mol. Cell* 64 (6) (2016) 1109–1116.
- [9] D. D'Amours, S. Desnoyers, I. D'Silva, G.G. Poirier, Poly(ADP-ribosylation) reactions in the regulation of nuclear functions, *Biochem. J.* 342 (Pt 2) (1999) 249–268.
- [10] M. De Vos, V. Schreiber, F. Dantzer, The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art, *Biochem. Pharmacol.* 84 (2) (2012) 137–146.
- [11] M.F. Langelier, T. Eiseemann, A.A. Riccio, J.M. Pascal, PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification, *Curr. Opin. Struct. Biol.* 53 (2018) 187–198.
- [12] R. Krishnakumar, W.L. Kraus, PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway, *Mol. Cell* 39 (5) (2010) 736–749.
- [13] B.A. Gibson, Y. Zhang, H. Jiang, K.M. Hussey, J.H. Shrimp, H. Lin, F. Schwede, Y. Yu, W.L. Kraus, Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation, *Science* 353 (6294) (2016) 45–50.
- [14] R. Gupta, Z. Liu, W.L. Kraus, PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes, *Genes Dev.* 31 (2) (2017) 101–126.
- [15] Z. Liu, W.L. Kraus, Catalytic-independent functions of PARP-1 determine Sox2 pioneer activity at intractable genomic loci, *Mol. Cell* 65 (4) (2017) 589–603 e9.
- [16] S.J. Hsiao, S. Smith, Tankyrase function at telomeres, spindle poles, and beyond, *Biochimie* 90 (1) (2008) 83–92.
- [17] P. Chang, M. Coughlin, T.J. Mitchison, Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function, *Nat. Cell Biol.* 7 (11) (2005) 1133–1139.
- [18] P. Chang, M. Coughlin, T.J. Mitchison, Interaction between Poly(ADP-ribose) and NuMA contributes to mitotic spindle pole assembly, *Mol. Biol. Cell* 20 (21) (2009) 4575–4585.
- [19] C. Boehler, L.R. Gauthier, O. Mortusewicz, D.S. Biard, J.M. Saliou, A. Bresson, S. Sanglier-Cianferani, S. Smith, V. Schreiber, F. Boussin, F. Dantzer, Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression, *Proc. Natl. Acad. Sci. U.S.A.* 108 (7) (2011) 2783–2788.
- [20] S. Vyas, M. Chesaroni-Cataldo, T. Todorova, Y.H. Huang, P. Chang, A systematic analysis of the PARP protein family identifies new functions critical for cell physiology, *Nat. Commun.* 4 (2013) 2240.
- [21] L. Palazzo, R. Della Monica, R. Visconti, V. Costanzo, D. Grieco, ATM controls proper mitotic spindle structure, *Cell Cycle* 13 (7) (2014) 1091–1100.
- [22] M. Yu, S. Schreek, C. Cerni, C. Schamberger, K. Lesniewicz, E. Poreba, J. Vervoorts, G. Walsemann, J. Grotzinger, E. Kremmer, Y. Mehraein, J. Mertsching, R. Kraft, M. Austen, J. Luscher-Firzlaff, B. Luscher, PARP-10, a novel Myc-interacting protein with poly(ADP-ribose) polymerase activity, inhibits transformation, *Oncogene* 24 (12) (2005) 1982–1993.

- [23] S.B. Bachmann, S.C. Frommel, R. Camicia, H.C. Winkler, R. Santoro, P.O. Hassa, DTX3L and ARTD9 inhibit IRF1 expression and mediate in cooperation with ARTD8 survival and proliferation of metastatic prostate cancer cells, *Mol. Cancer* 13 (2014) 125.
- [24] N. Li, Y. Zhang, X. Han, K. Liang, J. Wang, L. Feng, W. Wang, Z. Songyang, C. Lin, L. Yang, Y. Yu, J. Chen, Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth, *Genes Dev.* 29 (2) (2015) 157–170.
- [25] E.M. Schleicher, A.M. Galvan, Y. Imamura-Kawasawa, G.L. Moldovan, C.M. Nicolae, PARP10 promotes cellular proliferation and tumorigenesis by alleviating replication stress, *Nucleic Acids Res.* 46 (17) (2018) 8908–8916.
- [26] S.A. Andrabi, N.S. Kim, S.W. Yu, H. Wang, D.W. Koh, M. Sasaki, J.A. Klaus, T. Otsuka, Z. Zhang, R.C. Koehler, P.D. Hurn, G.G. Poirier, V.L. Dawson, T.M. Dawson, Poly(ADP-ribose) (PAR) polymer is a death signal, *Proc. Natl. Acad. Sci. U.S.A.* 103 (48) (2006) 18308–18313.
- [27] S.A. Andrabi, H.C. Kang, J.F. Haince, Y.I. Lee, J. Zhang, Z. Chi, A.B. West, R.C. Koehler, G.G. Poirier, T.M. Dawson, V.L. Dawson, Iduna protects the brain from glutamate excitotoxicity and stroke by interfering with poly(ADP-ribose) polymer-induced cell death, *Nat. Med.* 17 (6) (2011) 692–699.
- [28] Y. Wang, N.S. Kim, J.F. Haince, H.C. Kang, K.K. David, S.A. Andrabi, G.G. Poirier, V.L. Dawson, T.M. Dawson, Poly(ADP-ribose) (PAR) binding to apoptosis-inducing factor is critical for PAR polymerase-1-dependent cell death (parthanatos), *Sci. Signal* 4 (167) (2011) ra20.
- [29] X. Luo, W.L. Kraus, On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1, *Genes Dev.* 26 (5) (2012) 417–432.
- [30] L. Virag, A. Robaszekiewicz, J.M. Rodriguez-Vargas, F.J. Oliver, Poly(ADP-ribose) signaling in cell death, *Mol. Aspects Med.* 34 (6) (2013) 1153–1167.
- [31] N.C. Simon, K. Aktories, J.T. Barbieri, Novel bacterial ADP-ribosylating toxins: structure and function, *Nat. Rev. Microbiol.* 12 (9) (2014) 599–611.
- [32] C.V. Kuny, C.S. Sullivan, Virus-host interactions and the ARTD/PARP family of enzymes, *PLoS Pathog.* 12 (3) (2016) e1005453.
- [33] A.K.L. Leung, R.L. McPherson, D.E. Griffin, Macrodomein ADP-ribosylhydrolase and the pathogenesis of infectious diseases, *PLoS Pathog.* 14 (3) (2018) e1006864.
- [34] P.N. Brady, A. Goel, M.A. Johnson, Poly(ADP-Ribose) polymerases in host-pathogen interactions, inflammation, and immunity, *Microbiol. Mol. Biol. Rev.* 83 (1) (2019).
- [35] A.R. Fehr, G. Jankevicius, I. Ahel, S. Perlman, Viral macrodomains: unique mediators of viral replication and pathogenesis, *Trends Microbiol.* 26 (7) (2018) 598–610.
- [36] L. Palazzo, P. Mikolcovic, A. Mikoc, I. Ahel, ADP-ribosylation signalling and human disease, *Open biology* 9 (4) (2019) 190041.
- [37] C.J. Lord, A. Ashworth, PARP inhibitors: synthetic lethality in the clinic, *Science* 355 (6330) (2017) 1152–1158.
- [38] A. Ashworth, C.J. Lord, Synthetic lethal therapies for cancer: what's next after PARP inhibitors? *Nat. Rev. Clin. Oncol.* 15 (9) (2018) 564–576.
- [39] L. Palazzo, I. Ahel, PARPs in genome stability and signal transduction: implications for cancer therapy, *Biochem. Soc. Trans.* 46 (6) (2018) 1681–1695.
- [40] M.J. Pallen, A.C. Lam, N.J. Loman, A. McBride, An abundance of bacterial ADP-ribosyltransferases—implications for the origin of exotoxins and their human homologues, *Trends Microbiol.* 9 (7) (2001) 302–307 discussion 308.
- [41] S. Han, J.A. Tainer, The ARTT motif and a unified structural understanding of substrate recognition in ADP-ribosylating bacterial toxins and eukaryotic ADP-ribosyltransferases, *Int. J. Med. Microbiol.* 291 (6–7) (2002) 523–529.
- [42] M.O. Hottiger, P.O. Hassa, B. Luscher, H. Schuler, F. Koch-Nolte, Toward a unified nomenclature for mammalian ADP-ribosyltransferases, *Trends Biochem. Sci.* 35 (4) (2010) 208–219.
- [43] S. Vyas, I. Matic, L. Uchima, J. Rood, R. Zaja, R.T. Hay, I. Ahel, P. Chang, Family-wide analysis of poly(ADP-ribose) polymerase activity, *Nat. Commun.* 5 (2014) 4426.
- [44] P. Verheugd, M. Butepage, L. Eckei, B. Luscher, Players in ADP-ribosylation: readers and erasers, *Curr. Protein Pept. Sci.* 17 (7) (2016) 654–667.
- [45] M.S. Cohen, P. Chang, Insights into the biogenesis, function, and regulation of ADP-ribosylation, *Nat. Chem. Biol.* 14 (3) (2018) 236–243.
- [46] R.J. Collier, Diphtheria toxin: mode of action and structure, *Bacteriol. Rev.* 39 (1) (1975) 54–85.
- [47] D.M. Gill, The arrangement of subunits in cholera toxin, *Biochemistry* 15 (6) (1976) 1242–1248.
- [48] A.M. Pappenheimer Jr., D.M. Gill, Diphtheria, *Science* 182 (4110) (1973) 353–358.
- [49] D. Cassel, T. Pfeuffer, Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system, *Proc. Natl. Acad. Sci. U.S.A.* 75 (6) (1978) 2669–2673.
- [50] R.G. Zhang, D.L. Scott, M.L. Westbrook, S. Nance, B.D. Spangler, G.G. Shipley, E.M. Westbrook, The three-dimensional crystal structure of cholera toxin, *J. Mol. Biol.* 251 (4) (1995) 563–573.
- [51] C.E. Bell, D. Eisenberg, Crystal structure of diphtheria toxin bound to nicotinamide adenine dinucleotide, *Biochemistry* 35 (4) (1996) 1137–1149.
- [52] J.W. Peterson, Bacterial pathogenesis, Galveston (TX), in: S. Baron (Ed.), *Medical Microbiology*, 1996.
- [53] R.J. Collier, Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century, *Toxicon* 39 (11) (2001) 1793–1803.
- [54] K.P. Holbourn, C.C. Shone, K.R. Acharya, A family of killer toxins. Exploring the mechanism of ADP-ribosylating toxins, *FEBS J.* 273 (20) (2006) 4579–4593.
- [55] K. Aktories, Rho-modifying bacterial protein toxins, *Pathog. Dis.* 73 (9) (2015) ftv091.
- [56] Q. Deng, J.T. Barbieri, Modulation of host cell endocytosis by the type III cytotoxin. *Pseudomonas ExoS*, *Traffic* 9 (11) (2008) 1948–1957.
- [57] R.J. Fieldhouse, Z. Turgeon, D. White, A.R. Merrill, Cholera- and anthrax-like toxins are among several new ADP-ribosyltransferases, *PLoS Comput. Biol.* 6 (12) (2010) e1001029.
- [58] B. Feng, C. Liu, L. Shan, P. He, Protein ADP-ribosylation takes control in plant-bacterium interactions, *PLoS Pathog.* 12 (12) (2016) e1005941.
- [59] D.J. Wilson, Insights from genomics into bacterial pathogen populations, *PLoS Pathog.* 8 (9) (2012) e1002874.
- [60] L.B. Rice, The clinical consequences of antimicrobial resistance, *Curr. Opin. Microbiol.* 12 (5) (2009) 476–481.
- [61] P.M. Hawkey, A.M. Jones, The changing epidemiology of resistance, *J. Antimicrob. Chemother.* 64 (Suppl 1) (2009) i3–i10.
- [62] J. Davies, D. Davies, Origins and evolution of antibiotic resistance, *Microbiol. Mol. Biol. Rev.* 74 (3) (2010) 417–433.
- [63] A.E. Clatworthy, E. Pierson, D.T. Hung, Targeting virulence: a new paradigm for antimicrobial therapy, *Nat. Chem. Biol.* 3 (9) (2007) 541–548.
- [64] L. Cegelski, G.R. Marshall, G.R. Eldridge, S.J. Hultgren, The biology and future prospects of antivirulence therapies, *Nat. Rev. Microbiol.* 6 (1) (2008) 17–27.
- [65] L. Czaplewski, R. Bax, M. Clokie, M. Dawson, H. Fairhead, V.A. Fischetti, S. Foster, B.F. Gilmore, R.E. Hancock, D. Harper, I.R. Henderson, K. Hilpert, B.V. Jones, A. Kadioglu, D. Knowles, S. Olafsdottir, D. Payne, S. Projan, S. Shaunak, J. Silverman, C.M. Thomas, T.J. Trust, P. Warn, J.H. Rex, Alternatives to antibiotics—a pipeline portfolio review, *Lancet Infect. Dis.* 16 (2) (2016) 239–251.
- [66] N. Tuyiringire, D. Tusubira, J.P. Munyampundu, C.U. Tolo, C.M. Muvunyi, P.E. Ogwang, Application of metabolomics to drug discovery and understanding the mechanisms of action of medicinal plants with anti-tuberculosis activity, *Clin. Transl. Med.* 7 (1) (2018) 29.
- [67] G. Grimaldi, D. Corda, G. Catara, From toxins to mammalian enzymes: the diversity of mono-ADP-ribosylation, *Front. Biosci. (Landmark Ed)* 20 (2015) 389–404.
- [68] G. Fabrizio, E.S. Scarpa, M. Di Girolamo, State of the art of protein mono-ADP-ribosylation: biological role and therapeutic potential, *Front. Biosci. (Landmark Ed)* 20 (2015) 405–430.
- [69] J.D. Steffen, J.R. Brody, R.S. Armen, J.M. Pascal, Structural implications for selective targeting of PARPs, *Front. Oncol.* 3 (2013) 301.
- [70] S. Vyas, P. Chang, New PARP targets for cancer therapy, *Nat. Rev. Cancer* 14 (7) (2014) 502–509.
- [71] G. Grimaldi, G. Catara, L. Palazzo, A. Corteggio, C. Valente, D. Corda, PARPs and PAR as novel pharmacological targets for the treatment of stress granule-associated disorders, *Biochem. Pharmacol.* (2019).
- [72] P. Chambon, J.D. Weill, P. Mandel, Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme, *Biochem. Biophys. Res. Commun.* 11 (1963) 39–43.
- [73] R.J. Collier, A.M. Pappenheimer Jr., Studies on the mode of action of diphtheria toxin. I. Phosphorylated intermediates in normal and intoxicated hela cells, *J. Exp. Med.* 120 (1964) 1007–1018.
- [74] M.F. Langelier, J.L. Planck, S. Roy, J.M. Pascal, Structural basis for DNA damage-dependent poly(ADP-ribosylation) by human PARP-1, *Science* 336 (6082) (2012) 728–732.
- [75] S. Jungmichel, F. Rosenthal, M. Altmeyer, J. Lukas, M.O. Hottiger, M.L. Nielsen, Proteome-wide identification of poly(ADP-Ribosylation) targets in different genotoxic stress responses, *Mol. Cell* 52 (2) (2013) 272–285.
- [76] I. Gibbs-Seymour, P. Fontana, J.G.M. Rack, I. Ahel, HPF1/C4orf27 Is a PARP-1-interacting protein that regulates PARP-1 ADP-ribosylation activity, *Mol. Cell* 62 (3) (2016) 432–442.
- [77] J.M. Pascal, The comings and goings of PARP-1 in response to DNA damage, *DNA Repair (Amst)* 71 (2018) 177–182.
- [78] B. Luscher, M. Butepage, L. Eckei, S. Krieg, P. Verheugd, B.H. Shilton, ADP-ribosylation, a multifaceted posttranslational modification involved in the control of cell physiology in health and disease, *Chem. Rev.* 118 (3) (2018) 1092–1136.
- [79] P. Bai, C. Canto, The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease, *Cell Metab.* 16 (3) (2012) 290–295.
- [80] J. Marton, T. Fodor, L. Nagy, A. Vida, G. Kis, A. Brunyanski, M. Antal, B. Luscher, P. Bai, PARP10 (ARTD10) modulates mitochondrial function, *PLoS One* 13 (1) (2018) e0187789.
- [81] A. Burkle, C. Brabeck, J. Diefenbach, S. Beneke, The emerging role of poly(ADP-ribose) polymerase-1 in longevity, *Int. J. Biochem. Cell Biol.* 37 (5) (2005) 1043–1053.
- [82] S. Atasheva, M. Akhrymuk, E.I. Frolova, I. Frolov, New PARP gene with an anti-alphavirus function, *J. Virol.* 86 (15) (2012) 8147–8160.
- [83] S. Atasheva, E.I. Frolova, I. Frolov, Interferon-stimulated poly(ADP-Ribose) polymerases are potent inhibitors of cellular translation and virus replication, *J. Virol.* 88 (4) (2014) 2116–2130.
- [84] L. Li, H. Zhao, P. Liu, C. Li, N. Quanxin, X. Ji, N. Sun, P. Du, C.F. Qin, N. Lu, G. Cheng, PARP12 suppresses Zika virus infection through PARP-dependent degradation of NS1 and NS3 viral proteins, *Sci. Signal* 11 (535) (2018).
- [85] A.K. Leung, S. Vyas, J.E. Rood, A. Bhutkar, P.A. Sharp, P. Chang, Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm, *Mol. Cell* 42 (4) (2011) 489–499.
- [86] M. Jwa, P. Chang, PARP16 is a tail-anchored endoplasmic reticulum protein required for the PERK- and IRE1 α -mediated unfolded protein response, *Nat. Cell Biol.* 14 (11) (2012) 1223–1230.
- [87] S. Di Paola, M. Micaroni, G. Di Tullio, R. Buccione, M. Di Girolamo, PARP16/ARTD15 is a novel endoplasmic-reticulum-associated mono-ADP-ribosyltransferase that interacts with, and modifies karyopherin-ss1, *PLoS One* 7 (6) (2012) e37352.
- [88] G. Fabrizio, S. Di Paola, A. Stilla, M. Giannotta, C. Ruggiero, S. Menzel, F. Koch-

- Nolte, M. Sallase, M. Di Girolamo, ARTC1-mediated ADP-ribosylation of GRP78/BiP: a new player in endoplasmic-reticulum stress responses, *Cell. Mol. Life Sci.* 72 (6) (2015) 1209–1225.
- [89] G. Catara, G. Grimaldi, L. Schembri, D. Spano, G. Turacchio, M. Lo Monte, A.R. Beccari, C. Valente, D. Corda, PARP1-produced poly-ADP-ribose causes the PARP12 translocation to stress granules and impairment of Golgi complex functions, *Sci. Rep.* 7 (1) (2017) 14035.
- [90] G. Grimaldi, D. Corda, ADP-ribosylation and intracellular traffic: an emerging role for PARP enzymes, *Biochem. Soc. Trans.* 47 (1) (2019) 357–370.
- [91] M. Butepage, L. Eckei, P. Verheugd, B. Luscher, Intracellular mMono-ADP-ribosylation in signaling and disease, *Cells* 4 (4) (2015) 569–595.
- [92] R. Sharifi, R. Morra, C.D. 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, I. Ahel, Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease, *EMBO J.* 32 (9) (2013) 1225–1237.
- [93] P. Narne, V. Pandey, P.K. Simhadri, P.B. Phanithi, Poly(ADP-ribose)polymerase-1 hyperactivation in neurodegenerative diseases: the death knell tolls for neurons, *Semin. Cell Dev. Biol.* 63 (2017) 154–166.
- [94] K. Danhauser, B. Alhaddad, C. Makowski, D. Piekutowska-Abramczuk, S. Syrbe, N. Gomez-Ospina, M.A. Manning, A. Kostera-Pruszczyk, C. Krahn-Peper, R. Berutti, R. Kovacs-Nagy, M. Gusic, E. Graf, L. Laugwitz, M. Roblitz, A. Wroblewski, H. Hartmann, A.M. Das, E. Bultmann, F. Fang, M. Xu, U.A. Schatz, D. Karall, H. Zellner, E. Haberlondt, R.G. Feichtinger, J.A. Mayr, T. Meitinger, H. Prokisch, T.M. Strom, R. Ploski, G.F. Hoffmann, M. Pronicki, P.E. Bonnen, S. Morlot, T.B. Haack, Bi-allelic ADPRHL2 mutations cause neurodegeneration with developmental delay ataxia, and axonal neuropathy, *Am. J. Hum. Genet.* 103 (5) (2018) 817–825.
- [95] S.G. Ghosh, K. Becker, H. Huang, T. Dixon-Salazar, G. Chai, V. Salpietro, L. Al-Gazali, Q. Waisfisz, H. Wang, K.K. Vaux, V. Stanley, A. Manole, U. Akpulat, M.M. Weiss, S. Efthymiou, M.G. Hanna, C. Minetti, P. Striano, L. Pisciotto, E. De Grandis, J. Altmuller, P. Nurnberg, H. Thiele, U. Yis, T.D. Okur, A.I. Polat, N. Amiri, M. Doosti, E.G. Karimani, M.B. Toosi, G. Haddad, M. Karakaya, B. Wirth, J.M. van Hagen, N.I. Wolf, R. Maroofian, H. Houlden, S. Cirak, J.G. Gleeson, Biallelic mMutations in ADPRHL2, encoding ADP-ribosylhydrolase 3, lead to a degenerative pediatric stress-induced epileptic ataxia syndrome, *Am. J. Hum. Genet.* 103 (3) (2018) 431–439.
- [96] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, *Annu. Rev. Pathol.* 5 (2010) 253–295.
- [97] D. Corda, M. Di Girolamo, Functional aspects of protein mono-ADP-ribosylation, *EMBO J.* 22 (9) (2003) 1953–1958.
- [98] J.F. Rippmann, K. Damm, 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) (2002) 217–224.
- [99] S. Han, A.S. Arvai, S.B. Clancy, J.A. Tainer, Crystal structure and novel recognition motif of rho ADP-ribosylating C3 exoenzyme from *Clostridium botulinum*: structural insights for recognition specificity and catalysis, *J. Mol. Biol.* 305 (1) (2001) 95–107.
- [100] B.A. Wilson, K.A. Reich, B.R. Weinstein, R.J. Collier, Active-site mutations of diphtheria toxin: effects of replacing glutamic acid-148 with aspartic acid, glutamine, or serine, *Biochemistry* 29 (37) (1990) 8643–8651.
- [101] S.F. Carroll, R.J. Collier, NAD binding site of diphtheria toxin: identification of a residue within the nicotinamide subsite by photochemical modification with NAD, *Proc. Natl. Acad. Sci. U.S.A.* 81 (11) (1984) 3307–3311.
- [102] C.M. Douglas, R.J. Collier, Exotoxin A of *Pseudomonas aeruginosa*: substitution of glutamic acid 553 with aspartic acid drastically reduces toxicity and enzymatic activity, *J. Bacteriol.* 169 (11) (1987) 4967–4971.
- [103] G. de Murcia, V. Schreiber, M. Molinete, B. Saulier, O. Poch, M. Masson, C. Niedergang, J. Menissier de Murcia, Structure and function of poly(ADP-ribose) polymerase, *Mol. Cell. Biochem.* 138 (1–2) (1994) 15–24.
- [104] K. Hayashi, M. Tanaka, T. Shimada, M. Miwa, T. Sugimura, Size and shape of poly(ADP-ribose): examination by gel filtration, gel electrophoresis and electron microscopy, *Biochem. Biophys. Res. Commun.* 112 (1) (1983) 102–107.
- [105] A. Ruf, V. Rolli, G. de Murcia, G.E. Schulz, The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis, *J. Mol. Biol.* 278 (1) (1998) 57–65.
- [106] Q. Chen, M.A. Kassab, F. Dantzer, X. Yu, PARP2 mediates branched poly ADP-ribosylation in response to DNA damage, *Nat. Commun.* 9 (1) (2018) 3233.
- [107] T. Karlberg, M. Klepsch, A.G. Thorsell, C.D. Andersson, A. Linusson, H. Schuler, Structural basis for lack of ADP-ribosyltransferase activity in poly(ADP-ribose) polymerase-13/zinc finger antiviral protein, *J. Biol. Chem.* 290 (12) (2015) 7336–7344.
- [108] C.S. Yang, K. Jividen, A. Spencer, N. Dworak, L. Ni, L.T. Oostdyk, M. Chatterjee, B. Kusmider, B. Reon, M. Parlak, V. Gorbunova, T. Abbas, E. Jeffery, N.E. Sherman, B.M. Paschal, Ubiquitin modification by the E3 ligase/ADP-ribosyltransferase Dtx3L/Parp9, *Mol. Cell* 66 (4) (2017) 503–516 e5.
- [109] I.J. Okazaki, J. Moss, Characterization of glycosylphosphatidylinositol-anchored, secreted, and intracellular vertebrate mono-ADP-ribosyltransferases, *Annu. Rev. Nutr.* 19 (1999) 485–509.
- [110] G. Glowacki, R. Braren, K. Firner, M. Nissen, M. Kuhl, P. Reche, F. Bazan, M. Cetkovic-Cvrlje, E. Leiter, F. Haag, F. Koch-Nolte, The family of toxin-related ecto-ADP-ribosyltransferases in humans and the mouse, *Protein Sci.* 11 (7) (2002) 1657–1670.
- [111] M. Seman, S. Adriouch, F. Scheuplein, C. Krebs, D. Freese, G. Glowacki, P. Deterre, F. Haag, F. Koch-Nolte, NAD-induced T cell death: ADP-ribosylation of cell surface proteins by ART2 activates the cytolytic P2X7 purinoceptor, *Immunity* 19 (4) (2003) 571–582.
- [112] M. Seman, S. Adriouch, F. Haag, F. Koch-Nolte, Ecto-ADP-ribosyltransferases (ARTs): emerging actors in cell communication and signaling, *Curr. Med. Chem.* 11 (7) (2004) 857–872.
- [113] F. Koch-Nolte, S. Kernstock, C. Mueller-Dieckmann, M.S. Weiss, F. Haag, Mammalian ADP-ribosyltransferases and ADP-ribosylhydrolases, *Front. Biosci.* 13 (2008) 6716–6729.
- [114] S. Laing, M. Unger, F. Koch-Nolte, F. Haag, ADP-ribosylation of arginine, *Amino Acids* 41 (2) (2011) 257–269.
- [115] K. Crawford, J.J. Bonfiglio, A. Mikoc, I. Matic, I. Ahel, Specificity of reversible ADP-ribosylation and regulation of cellular processes, *Crit. Rev. Biochem. Mol. Biol.* 53 (1) (2018) 64–82.
- [116] M. Leutert, S. Menzel, R. Braren, B. Rissiek, A.K. Hopp, K. Nowak, L. Bisceglie, P. Gehrig, H. Li, A. Zolkiewska, F. Koch-Nolte, M.O. Hottiger, Proteomic characterization of the heart and skeletal muscle reveals widespread Arginine ADP-ribosylation by the ARTC1 ectoenzyme, *Cell Rep.* 24 (7) (2018) 1916–1929 e5.
- [117] I. Matic, I. Ahel, R.T. Hay, Reanalysis of phosphoproteomics data uncovers ADP-ribosylation sites, *Nat. Methods* 9 (8) (2012) 771–772.
- [118] R. Maitello, M. Leutert, S. Jungmichel, V. Bilan, S.C. Larsen, C. Young, M.O. Hottiger, M.L. Nielsen, Proteome-wide identification of the endogenous ADP-ribosylome of mammalian cells and tissue, *Nat. Commun.* 7 (2016) 12917.
- [119] R. Lupi, D. Corda, M. Di Girolamo, Endogenous ADP-ribosylation of the G protein beta subunit prevents the inhibition of type 1 adenylyl cyclase, *J. Biol. Chem.* 275 (13) (2000) 9418–9424.
- [120] M. Watanabe, T. Takamura-Enya, T. Kanazawa, Y. Totsuka, Y. Matsushima-Hibiya, K. Koyama, T. Sugimura, K. Wakabayashi, Mono(ADP-ribosyl)ation of DNA by apoptosis-inducing protein, pterisin, *Nucl. Acids Res. (Suppl.)* (2002) 243–244.
- [121] I. Carpusca, T. Jank, K. Aktories, *Bacillus sphaericus* mosquitocidal toxin (MTX) and pterisin: the enigmatic offspring from the family of ADP-ribosyltransferases, *Mol. Microbiol.* 62 (3) (2006) 621–630.
- [122] T. Nakano, Y. Matsushima-Hibiya, M. Yamamoto, A. Takahashi-Nakaguchi, H. Fukuda, M. Ono, T. Takamura-Enya, H. Kinashi, Y. Totsuka, ADP-ribosylation of guanosine by SCO5461 protein secreted from *Streptomyces coelicolor*, *Toxicol.* 63 (2013) 55–63.
- [123] B. Lyons, R. Ravulapalli, J. Lanoue, M.R. Lugo, D. Dutta, S. Carlin, A.R. Merrill, Scabin, a Novel DNA-acting ADP-ribosyltransferase from *Streptomyces scabies*, *J. Biol. Chem.* 291 (21) (2016) 11198–12125.
- [124] T. Nakano, Y. Matsushima-Hibiya, M. Yamamoto, S. Enomoto, Y. Matsumoto, Y. Totsuka, M. Watanabe, T. Sugimura, K. Wakabayashi, Purification and molecular cloning of a DNA ADP-ribosylating protein, CARP-1, from the edible clam *Meretrix lamarckii*, *Proc. Natl. Acad. Sci. U.S.A.* 103 (37) (2006) 13652–13657.
- [125] M. Yamamoto, T. Nakano, Y. Matsushima-Hibiya, Y. Totsuka, A. Takahashi-Nakaguchi, Y. Matsumoto, T. Sugimura, K. Wakabayashi, Molecular cloning of apoptosis-inducing Pterisin-like proteins, from two species of white butterfly *Pieris melete* and *Aporia crataegi*, *Comput. Biochem. Physiol. B Biochem. Mol. Biol.* 154 (3) (2009) 326–333.
- [126] I. Talhaoui, N.A. Lebedeva, G. Zarkovic, C. Saint-Pierre, M.M. Kutuzov, M.V. Sukhanova, B.T. Matkarimov, D. Gasparutto, M.K. Saparbaev, O.I. Lavrik, A.A. Ishchenko, Poly(ADP-ribose) polymerases covalently modify strand break termini in DNA fragments in vitro, *Nucleic Acids Res.* 44 (19) (2016) 9279–9295.
- [127] E.A. Belousova, C.A. Ishchenko, O.I. Lavrik, Dna is a New Target of Parp3, *Sci. Rep.* 8 (1) (2018) 4176.
- [128] G. Zarkovic, E.A. Belousova, I. Talhaoui, C. Saint-Pierre, M.M. Kutuzov, B.T. Matkarimov, D. Biard, D. Gasparutto, O.I. Lavrik, A.A. Ishchenko, Characterization of DNA ADP-ribosyltransferase activities of PARP2 and PARP3: new insights into DNA ADP-ribosylation, *Nucl. Acids Res.* 46 (5) (2018) 2417–2431.
- [129] S.L. Spinelli, R. Kierzek, D.H. Turner, E.M. Phizicky, Transient ADP-ribosylation of a 2'-phosphate implicated in its removal from ligated tRNA during splicing in yeast, *J. Biol. Chem.* 274 (5) (1999) 2637–2644.
- [130] A. Munir, A. Banerjee, S. Shuman, NAD+ dependent synthesis of a 5'-phospho-ADP-ribosylated RNA/DNA cap by RNA 2'-phosphotransferase Tpt1, *Nucl. Acids Res.* 46 (18) (2018) 9617–9624.
- [131] A. Banerjee, A. Munir, L. Abdullahu, M.J. Damha, Y. Goldgur, S. Shuman, Structure of tRNA splicing enzyme Tpt1 illuminates the mechanism of RNA 2'-PO4 recognition and ADP-ribosylation, *Nat. Commun.* 10 (1) (2019) 218.
- [132] M.A. Steiger, R. Kierzek, D.H. Turner, E.M. Phizicky, Substrate recognition by a yeast 2'-phosphotransferase involved in tRNA splicing and by its *Escherichia coli* homolog, *Biochemistry* 40 (46) (2001) 14098–14105.
- [133] D. Munnur, E. Bartlett, P. Mikolčević, I.T. Kirby, J.G. Matthias Rack, A. Mikoč, M.S. Cohen, I. Ahel, Reversible ADP-ribosylation of RNA, *Nucl. Acids Res.* (2019).
- [134] S. Quan, H. Venter, E.R. Dabbs, Ribosylative inactivation of rifampin by *Mycobacterium smegmatis* is a principal contributor to its low susceptibility to this antibiotic, *Antimicrob. Agents Chemother.* 41 (11) (1997) 2456–2460.
- [135] S. Quan, T. Imai, Y. Mikami, K. Yazawa, E.R. Dabbs, N. Morisaki, S. Iwasaki, Y. Hashimoto, K. Furihata, ADP-ribosylation as an intermediate step in inactivation of rifampin by a mycobacterial gene, *Antimicrob. Agents Chemother.* 43 (1) (1999) 181–184.
- [136] H. Tsuge, M. Nagahama, M. Oda, S. Iwamoto, H. Utsunomiya, V.E. Marquez, N. Katunuma, M. Nishizawa, J. Sakurai, Structural basis of actin recognition and arginine ADP-ribosylation by *Clostridium perfringens* iota-toxin, *Proc. Natl. Acad. Sci. U.S.A.* 105 (21) (2008) 7399–7404.
- [137] A.E. Lang, G. Schmidt, A. Schlosser, T.D. Hey, I.M. Larrinua, J.J. Sheets, H.G. Mannherz, K. Aktories, Photolabidus luminescens toxins ADP-ribosylate actin and RhoA to force actin clustering, *Science* 327 (5969) (2010) 1139–1142.

- [138] T. Tsurumura, Y. Tsumori, H. Qiu, M. Oda, J. Sakurai, M. Nagahama, H. Tsuge, Arginine ADP-ribosylation mechanism based on structural snapshots of iota-toxin and actin complex, *Proc. Natl. Acad. Sci. U.S.A.* 110 (11) (2013) 4267–4272.
- [139] Y. Tamamura, K. Tanaka, I. Uchida, Characterization of pertussis-like toxin from *Salmonella* spp. that catalyzes ADP-ribosylation of G proteins, *Sci. Rep.* 7 (1) (2017) 2653.
- [140] J. Ebeling, A. Funfhaus, H. Knispel, D. Krška, R. Ravulapalli, K.A. Heney, M.R. Lugo, A.R. Merrill, E. Genersch, Characterization of the toxin Plx2A, a RhoA-targeting ADP-ribosyltransferase produced by the honey bee pathogen *Paenibacillus larvae*, *Environ. Microbiol.* 19 (12) (2017) 5100–5116.
- [141] J.C. Young, A. Clements, A.E. Lang, J.A. Garnett, D. Munera, A. Arbeloa, J. Pearson, E.L. Hartland, S.J. Matthews, A. Mousnier, D.J. Barry, M. Way, A. Schlosser, K. Aktories, G. Frankel, The *Escherichia coli* effector Src kinase activity via amidation and ADP ribosylation, *Nat. Commun.* 5 (2014) 5887.
- [142] D.J. Pollard, C.N. Berger, E.C. So, L. Yu, K. Hadavizadeh, P. Jennings, E.W. Tate, J.S. Choudhary, G. Frankel, Broad-spectrum regulation of nonreceptor tyrosine kinases by the bacterial ADP-ribosyltransferase EspJ, *mBio* 9 (2) (2018).
- [143] O. Leidecker, J.J. Bonfiglio, T. Colby, Q. Zhang, I. Atanassov, R. Zaja, L. Palazzo, A. Stockum, I. Ahel, I. Matic, Serine is a new target residue for endogenous ADP-ribosylation on histones, *Nat. Chem. Biol.* 12 (12) (2016) 998–1000.
- [144] L. Palazzo, O. Leidecker, E. Prokhorova, H. Dauben, I. Matic, I. Ahel, Serine is the major residue for ADP-ribosylation upon DNA damage, *eLife* 7 (2018).
- [145] S.C. Larsen, I.A. Hendriks, D. Lyon, L.J. Jensen, M.L. Nielsen, Systems-wide analysis of serine ADP-ribosylation reveals widespread occurrence and site-specific overlap with phosphorylation, *Cell Rep.* 24 (9) (2018) 2493–2505 e4.
- [146] D.M. Leslie Pedrioli, M. Leutert, V. Bilan, K. Nowak, K. Gunasekera, E. Ferrari, R. Imhof, L. Malmstrom, M.O. Hottiger, Comprehensive ADP-ribosylome analysis identifies tyrosine as an ADP-ribose acceptor site, *EMBO Rep.* 19 (8) (2018).
- [147] E. Bartlett, J.J. Bonfiglio, E. Prokhorova, T. Colby, F. Zobel, I. Ahel, I. Matic, Interplay of histone marks with serine ADP-ribosylation, *Cell Rep.* 24 (13) (2018) 3488–3502 e5.
- [148] Z. Tao, P. Gao, H.W. Liu, Identification of the ADP-ribosylation sites in the PARP-1 automodification domain: analysis and implications, *J. Am. Chem. Soc.* 131 (40) (2009) 14258–14260.
- [149] Y. Zhang, J. Wang, M. Ding, Y. Yu, Site-specific characterization of the Asp- and Glu-ADP-ribosylated proteome, *Nat. Methods* 10 (10) (2013) 981–984.
- [150] S. Messner, M. Altmeyer, H. Zhao, A. Pozivil, B. Roschitzki, P. Gehrig, D. Rutishauser, D. Huang, A. Cafisch, M.O. Hottiger, PARP1 ADP-ribosylates lysine residues of the core histone tails, *Nucl. Acids Res.* 38 (19) (2010) 6350–6362.
- [151] I.A. Hendriks, S.C. Larsen, M.L. Nielsen, An advanced strategy for comprehensive profiling of ADP-ribosylation sites using mass spectrometry-based proteomics, *Mol. Cell. Proteomics* (2019).
- [152] J.J. Bonfiglio, P. Fontana, Q. Zhang, T. Colby, I. Gibbs-Seymour, I. Atanassov, E. Bartlett, R. Zaja, I. Ahel, I. Matic, Serine ADP-ribosylation depends on HPF1, *Mol. Cell* 65 (5) (2017) 932–940 e6.
- [153] S. Guettler, J. LaRose, E. Petsalaki, G. Gish, A. Scotter, T. Pawson, R. Rottapel, F. Sicheri, Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease, *Cell* 147 (6) (2011) 1340–1354.
- [154] T. Eiseemann, M. McCauley, M.F. Langelier, K. Gupta, S. Roy, G.D. Van Duyn, J.M. Pascal, Tankyrase-1 ankyrin repeats form an adaptable binding platform for targets of ADP-ribose modification, *Structure* 24 (10) (2016) 1679–1692.
- [155] V.A. Kickhoefer, A.C. Siva, N.L. Kedersha, E.M. Inman, C. Ruland, M. Streuli, L.H. Rome, The 193-kD vault protein, VPAP, is a novel poly(ADP-ribose) polymerase, *J. Cell Biol.* 146 (5) (1999) 917–928.
- [156] E. Barkauskaite, G. Jankevicius, I. Ahel, Structures and mechanisms of enzymes employed in the synthesis and degradation of PARP-dependent protein ADP-ribosylation, *Mol. Cell* 58 (6) (2015) 935–946.
- [157] J. O'Sullivan, M. Tedim Ferreira, J.P. Gagne, A.K. Sharma, M.J. Hendzel, J.Y. Masson, G.G. Poirier, Emerging roles of eraser enzymes in the dynamic control of protein ADP-ribosylation, *Nature Commun.* 10 (1) (2019) 1182.
- [158] V.R. Moure, F.F. Costa, L.M. Cruz, F.O. Pedrosa, E.M. Souza, X.D. Li, F. Winkler, L.F. Huergo, Regulation of nitrogenase by reversible mono-ADP-ribosylation, *Curr. Top. Microbiol. Immunol.* 384 (2015) 89–106.
- [159] C.L. Berthold, H. Wang, S. Nordlund, M. Hogbom, Mechanism of ADP-ribosylation removal revealed by the structure and ligand complexes of the dimanganese mono-ADP-ribosylhydrolase DraG, *Proc. Natl. Acad. Sci. U.S.A.* 106 (34) (2009) 14247–14252.
- [160] C. Mueller-Dieckmann, S. Kernstock, M. Lisurek, J.P. von Kries, F. Haag, M.S. Weiss, F. Koch-Nolte, The structure of human ADP-ribosylhydrolase 3 (ARH3) provides insights into the reversibility of protein ADP-ribosylation, *Proc. Natl. Acad. Sci. U.S.A.* 103 (41) (2006) 15026–15031.
- [161] M. Mashimo, J. Kato, J. Moss, ADP-ribosyl-acceptor hydrolase 3 regulates poly(ADP-ribose) degradation and cell death during oxidative stress, *Proc. Natl. Acad. Sci. U.S.A.* 110 (47) (2013) 18964–18969.
- [162] M. Mashimo, J. Kato, J. Moss, Structure and function of the ARH family of ADP-ribosyl-acceptor hydrolases, *DNA Repair (Amst)* 23 (2014) 88–94.
- [163] M. Mashimo, J. Moss, Functional role of ADP-ribosyl-acceptor hydrolase 3 in poly(ADP-ribose) polymerase-1 response to oxidative stress, *Curr. Protein Pept. Sci.* 17 (7) (2016) 633–640.
- [164] P. Fontana, J.J. Bonfiglio, L. Palazzo, E. Bartlett, I. Matic, I. Ahel, Serine ADP-ribosylation reversal by the hydrolase ARH3, *eLife* 6 (2017).
- [165] J. Abplanalp, M. Leutert, E. Frugier, K. Nowak, R. Feurer, J. Kato, H.V.A. Kistemaker, D.V. Filippov, J. Moss, A. Cafisch, M.O. Hottiger, Proteomic analyses identify ARH3 as a serine mono-ADP-ribosylhydrolase, *Nat. Commun.* 8 (1) (2017) 2055.
- [166] J.G.M. Rack, A. Ariza, B.S. Drown, C. Henfrey, E. Bartlett, T. Shirai, P.J. Hergenrother, I. Ahel, (ADP-ribosyl)hydrolases: structural basis for differential substrate recognition and inhibition, *Cell Chem. Biol.* 25 (12) (2018) 1533–1546 e12.
- [167] X. Bu, J. Kato, J. Moss, Emerging roles of ADP-ribosyl-acceptor hydrolases (ARHs) in tumorigenesis and cell death pathways, *Biochem. Pharmacol.* (2018).
- [168] P.W. Ludden, Reversible ADP-ribosylation as a mechanism of enzyme regulation in prokaryotes, *Mol. Cell. Biochem.* 138 (1–2) (1994) 123–129.
- [169] J. Kato, J. Zhu, C. Liu, J. Moss, Enhanced sensitivity to cholera toxin in ADP-ribosylarginine hydrolase-deficient mice, *Mol. Cell. Biol.* 27 (15) (2007) 5534–5543.
- [170] K. Watanabe, J. Kato, J. Zhu, H. Oda, H. Ishiwata-Endo, J. Moss, Enhanced sensitivity to cholera toxin in female ADP-ribosylarginine hydrolase (ARH1)-deficient mice, *PLoS One* 13 (11) (2018) e0207693.
- [171] J.G. Rack, D. Perina, I. Ahel, Macromolecules: structure, function, evolution, and catalytic activities, *Annu. Rev. Biochem.* 85 (2016) 431–454.
- [172] K.L. Feijs, A.H. Forst, P. Verheugd, B. Luscher, Macromolecule-containing proteins: regulating new intracellular functions of mono(ADP-ribosyl)ation, *Nat. Rev. Mol. Cell Biol.* 14 (7) (2013) 443–451.
- [173] N. Dani, A. Stilla, A. Marchegiani, A. Tamburro, S. Till, A.G. Ladurner, D. Corda, 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. U.S.A.* 106 (11) (2009) 4243–4248.
- [174] G. Bartolomei, M. Leutert, M. Manzo, T. Baubec, M.O. Hottiger, Analysis of chromatin ADP-ribosylation at the genome-wide level and at specific loci by ADPr-ChAP, *Mol. Cell* 61 (3) (2016) 474–485.
- [175] V. Bilan, N. Selevsek, H.A.V. Kistemaker, J. Abplanalp, R. Feurer, D.V. Filippov, M.O. Hottiger, New quantitative mass spectrometry approaches reveal different ADP-ribosylation phases dependent on the levels of oxidative stress, *Mol. Cell. Proteomics* 16 (5) (2017) 949–958.
- [176] D. Slade, M.S. Dunstan, E. Barkauskaite, R. Weston, P. Lafite, N. Dixon, M. Ahel, D. Leys, I. Ahel, The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase, *Nature* 477 (7366) (2011) 616–620.
- [177] M.S. Dunstan, E. Barkauskaite, P. Lafite, C.E. Knezevic, A. Brassington, M. Ahel, P.J. Hergenrother, D. Leys, I. Ahel, Structure and mechanism of a canonical poly(ADP-ribose) glycohydrolase, *Nat. Commun.* 3 (2012) 878.
- [178] G.I. Karras, G. Kustatscher, H.R. Buhecha, M.D. Allen, C. Pugieux, F. Sait, M. Bycroft, A.G. Ladurner, The macro domain is an ADP-ribose binding module, *EMBO J.* 24 (11) (2005) 1911–1920.
- [179] G. Jankevicius, M. Hassler, B. Golia, V. Rybin, M. Zacharias, G. Timinsky, A.G. Ladurner, A family of macrodomain proteins reverses cellular mono-ADP-ribosylation, *Nat. Struct. Mol. Biol.* 20 (4) (2013) 508–514.
- [180] F. Rosenthal, K.L. Feijs, E. Frugier, M. Bonalli, A.H. Forst, R. Imhof, H.C. Winkler, D. Fischer, A. Cafisch, P.O. Hassa, B. Luscher, M.O. Hottiger, Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases, *Nat. Struct. Mol. Biol.* 20 (4) (2013) 502–507.
- [181] J.G. Rack, R. Morra, E. Barkauskaite, R. Kraehenbuehl, A. Ariza, Y. Qu, M. Ortmayer, O. Leidecker, D.R. Cameron, I. Matic, A.Y. Peleg, D. Leys, A. Traven, I. Ahel, Identification of a class of protein ADP-ribosylating sirtuins in microbial pathogens, *Mol. Cell* 59 (2) (2015) 309–320.
- [182] J. Lalic, M. Posavec Marjanovic, L. Palazzo, D. Perina, I. Sabljic, R. Zaja, T. Colby, B. Plese, M. Halasz, G. Jankevicius, G. Bucca, M. Ahel, I. Matic, H. Cetkovic, M. Lucic, A. Mikoc, I. Ahel, Disruption of macrodomain protein SCO6735 increases antibiotic production in *Streptomyces coelicolor*, *J. Biol. Chem.* 291 (2016) 23175–23187.
- [183] A.G. Garcia-Saura, R. Zapata-Perez, J.F. Hidalgo, J. Cabanes, F. Gil-Ortiz, A. Sanchez-Ferrer, An uncharacterized FMA01619 protein from *Fusobacterium mortiferum* ATCC 9817 demonstrates that some bacterial macrodomains can also act as poly-ADP-ribosylhydrolases, *Sci. Rep.* 9 (1) (2019) 3230.
- [184] C.C. Cho, C.Y. Chien, Y.C. Chiu, M.H. Lin, C.H. Hsu, Structural and biochemical evidence supporting poly ADP-ribosylation in the bacterium *Deinococcus radiodurans*, *Nat. Commun.* 10 (1) (2019) 1491.
- [185] C. Li, Y. Debing, G. Jankevicius, J. Neyts, I. Ahel, B. Coutard, B. Canard, Viral macro domains reverse protein ADP-ribosylation, *J. Virol.* 90 (19) (2016) 8478–8486.
- [186] M.E. Grunewald, Y. Chen, C. Kuny, T. Maejima, R. Lease, D. Ferraris, M. Aikawa, C.S. Sullivan, S. Perlman, A.R. Fehr, The coronavirus macrodomain is required to prevent PARP-mediated inhibition of virus replication and enhancement of IFN expression, *PLoS Pathog.* 15 (5) (2019) e1007756.
- [187] E. Lemichez, J.T. Barbieri, General aspects and recent advances on bacterial protein toxins, *Cold Spring Harb. Perspect. Med.* 3 (2) (2013) a013573.
- [188] K.M. Krueger, J.T. Barbieri, The family of bacterial ADP-ribosylating exotoxins, *Clin. Microbiol. Rev.* 8 (1) (1995) 34–47.
- [189] N. Wettschreck, S. Offermanns, Mammalian G proteins and their cell type specific functions, *Physiol. Rev.* 85 (4) (2005) 1159–1204.
- [190] D.J. Chinnappen, H. Chinnappen, D. Saslowsky, W.I. Lencer, Rafting with cholera toxin: endocytosis and trafficking from plasma membrane to ER, *FEMS Microbiol. Lett.* 266 (2) (2007) 129–137.
- [191] J. Moss, S.H. Richardson, Activation of adenylate cyclase by heat-labile *Escherichia coli* enterotoxin Evidence for ADP-ribosyltransferase activity similar to that of cholera toxin, *J. Clin. Invest.* 62 (2) (1978) 281–285.
- [192] S. Kopic, J.P. Geibel, Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, V. cholerae and rotavirus infection, *Toxins (Basel)* 2 (8) (2010) 2132–2157.
- [193] P.P. Chang, J. Moss, E.M. Twiddy, R.K. Holmes, Type II heat-labile enterotoxin of *Escherichia coli* activates adenylate cyclase in human fibroblasts by ADP ribosylation, *Infect. Immun.* 55 (8) (1987) 1854–1858.

- [194] H.S. Kantor, P. Tao, C. Wisdom, Action of *Escherichia coli* enterotoxin: adenylate cyclase behavior of intestinal epithelial cells in culture, *Infect. Immun.* 9 (6) (1974) 1003–1010.
- [195] T. Katada, M. Ui, Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein, *Proc. Natl. Acad. Sci. U.S.A.* 79 (10) (1982) 3129–3133.
- [196] G.M. Bokoch, T. Katada, J.K. Northup, E.L. Hewlett, A.G. Gilman, Identification of the predominant substrate for ADP-ribosylation by islet activating protein, *J. Biol. Chem.* 258 (4) (1983) 2072–2075.
- [197] H. Kurose, T. Katada, T. Amano, M. Ui, Specific uncoupling by islet-activating protein, pertussis toxin, of negative signal transduction via alpha-adrenergic, cholinergic, and opiate receptors in neuroblastoma x glioma hybrid cells, *J. Biol. Chem.* 258 (8) (1983) 4870–4875.
- [198] I. Uchida, R. Ishihara, K. Tanaka, E. Hata, S. Makino, T. Kanno, S. Hatama, M. Kishima, M. Akiba, A. Watanabe, T. Kubota, *Salmonella enterica* serotype Typhimurium DT104 ArtA-dependent modification of pertussis toxin-sensitive G proteins in the presence of [32P]NAD, *Microbiology* 155 (Pt 11) (2009) 3710–3718.
- [199] L. Carlier, C. Koehler, D. Veggi, M. Pizsa, M. Soriani, R. Boelens, A.M. Bonvin, NMR resonance assignments of NarE, a putative ADP-ribosylating toxin from *Neisseria meningitidis*, *Biomol. NMR Assign* 5 (1) (2011) 35–38.
- [200] M. Valeri, V. Zurli, I. Ayala, A. Colanzi, L. Lapazio, D. Corda, M. Soriani, M. Pizsa, S. Rossi Paccani, The *neisseria meningitidis* ADP-ribosyltransferase narE enters human epithelial cells and disrupts epithelial monolayer integrity, *PLoS One* 10 (5) (2015) e0127614.
- [201] V. Masignani, E. Balducci, F. Di Marcello, S. Savino, D. Serruto, D. Veggi, S. Bambini, M. Scarselli, B. Arico, M. Comanducci, J. Adu-Bobie, M.M. Giuliani, R. Rappuoli, M. Pizsa, NarE: a novel ADP-ribosyltransferase from *Neisseria meningitidis*, *Mol. Microbiol.* 50 (3) (2003) 1055–1067.
- [202] V. Masignani, E. Balducci, D. Serruto, D. Veggi, B. Arico, M. Comanducci, M. Pizsa, R. Rappuoli, In silico identification of novel bacterial ADP-ribosyltransferases, *Int. J. Med. Microbiol.* 293 (7–8) (2004) 471–478.
- [203] L. Gonzalez Jr., R.H. Scheller, Regulation of membrane trafficking: structural insights from a Rab/effector complex, *Cell* 96 (6) (1999) 755–758.
- [204] H. Stenmark, Rab GTPases as coordinators of vesicle traffic, *Nat. Rev. Mol. Cell Biol.* 10 (8) (2009) 513–525.
- [205] S.R. Pfeffer, Rab GTPase regulation of membrane identity, *Curr. Opin. Cell Biol.* 25 (4) (2013) 414–419.
- [206] C. D'Souza-Schorey, P. Chavrier, ARF proteins: roles in membrane traffic and beyond, *Nat. Rev. Mol. Cell Biol.* 7 (5) (2006) 347–358.
- [207] A.B. Jaffe, A. Hall, Rho GTPases: biochemistry and biology, *Annu. Rev. Cell Dev. Biol.* 21 (2005) 247–269.
- [208] K. Rottner, J. Faix, S. Bogdan, S. Linder, E. Kerkhoff, Actin assembly mechanisms at a glance, *J. Cell Sci.* 130 (20) (2017) 3427–3435.
- [209] I. Kjos, K. Vestre, N.A. Guadagno, M. Borg Distefano, C. Progida, Rab and Arf proteins at the crossroad between membrane transport and cytoskeleton dynamics, *Biochim Biophys Acta Mol. Cell Res.* 1865 (10) (2018) 1397–1409.
- [210] K. Aktories, Bacterial protein toxins that modify host regulatory GTPases, *Nat. Rev. Microbiol.* 9 (7) (2011) 487–498.
- [211] K. Aktories, M. Barmann, I. Ohishi, S. Tsuyama, K.H. Jakobs, E. Habermann, Botulinum C2 toxin ADP-ribosylates actin, *Nature* 322 (6077) (1986) 390–392.
- [212] J. Vandekerckhove, B. Schering, M. Barmann, K. Aktories, Clostridium perfringens iota toxin ADP-ribosylates skeletal muscle actin in Arg-177, *FEBS Lett.* 225 (1–2) (1987) 48–52.
- [213] B.G. Stiles, K. Pradhan, J.M. Fleming, R.P. Samy, H. Barth, M.R. Popoff, Clostridium and bacillus binary enterotoxins: bad for the bowels, and eukaryotic being, *Toxins (Basel)* 6 (9) (2014) 2626–2656.
- [214] H. Hochmann, S. Pust, G. von Figura, K. Aktories, H. Barth, *Salmonella enterica* SpvB ADP-ribosylates actin at position arginine-177-characterization of the catalytic domain within the SpvB protein and a comparison to binary clostridial actin-ADP-ribosylating toxins, *Biochemistry* 45 (4) (2006) 1271–1277.
- [215] G. Suarez, J.C. Sierra, T.E. Erova, J. Sha, A.J. Horneman, A.K. Chopra, A type VI secretion system effector protein, VgrG1, from *Aeromonas hydrophila* that induces host cell toxicity by ADP ribosylation of actin, *J. Bacteriol.* 192 (1) (2010) 155–168.
- [216] D. Fehr, S.E. Burr, M. Gibert, J. d'Alayer, J. Frey, M.R. Popoff, *Aeromonas* exoenzyme T of *Aeromonas salmonicida* is a bifunctional protein that targets the host cytoskeleton, *J. Biol. Chem.* 282 (39) (2007) 28843–28852.
- [217] K. Aktories, C. Schwan, A.E. Lang, ADP-ribosylation and cross-linking of actin by bacterial protein toxins, *Handb. Exp. Pharmacol.* 235 (2017) 179–206.
- [218] C. Schwan, A.S. Kruppke, T. Nolke, L. Schumacher, F. Koch-Nolte, M. Kudryashev, H. Stahlberg, K. Aktories, Clostridium difficile toxin CDT hijacks microtubule organization and reroutes vesicle traffic to increase pathogen adherence, *Proc. Natl. Acad. Sci. U.S.A.* 111 (6) (2014) 2313–2318.
- [219] K. Aktories, U. Braun, S. Rosener, I. Just, A. Hall, The rho gene product expressed in *E. coli* is a substrate of botulinum ADP-ribosyltransferase C3, *Biochem. Biophys. Res. Commun.* 158 (1) (1989) 209–213.
- [220] E.J. Rubin, D.M. Gill, P. Boquet, M.R. Popoff, Functional modification of a 21-kilodalton G protein when ADP-ribosylated by exoenzyme C3 of Clostridium botulinum, *Mol. Cell. Biol.* 8 (1) (1988) 418–426.
- [221] C. Wilde, M. Vogelsang, K. Aktories, Rho-specific *Bacillus cereus* ADP-ribosyltransferase C3 cloning and characterization, *Biochemistry* 42 (32) (2003) 9694–9702.
- [222] P. Chardin, P. Boquet, P. Madaule, M.R. Popoff, E.J. Rubin, D.M. Gill, The mammalian G protein rhoC is ADP-ribosylated by Clostridium botulinum exoenzyme C3 and affects actin microfilaments in Vero cells, *EMBO J.* 8 (4) (1989) 1087–1092.
- [223] H.F. Paterson, A.J. Self, M.D. Garrett, I. Just, K. Aktories, A. Hall, Microinjection of recombinant p21rho induces rapid changes in cell morphology, *J. Cell Biol.* 111 (3) (1990) 1001–1007.
- [224] W. Wieggers, I. Just, H. Muller, A. Hellwig, P. Traub, K. Aktories, Alteration of the cytoskeleton of mammalian cells cultured in vitro by Clostridium botulinum C2 toxin and C3 ADP-ribosyltransferase, *Eur. J. Cell Biol.* 54 (2) (1991) 237–245.
- [225] A. Nusrat, M. Giry, J.R. Turner, S.P. Colgan, C.A. Parkos, D. Carnes, E. Lemichez, P. Boquet, J.L. Madara, Rho protein regulates tight junctions and perijunctional actin organization in polarized epithelia, *Proc. Natl. Acad. Sci. U.S.A.* 92 (23) (1995) 10629–10633.
- [226] S.M. Stamatovic, R.F. Keep, S.L. Kunkel, A.V. Andjelkovic, Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling via Rho and Rho kinase, *J. Cell Sci.* 116 (Pt 22) (2003) 4615–4628.
- [227] E. Caron, A. Hall, Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases, *Science* 282 (5394) (1998) 1717–1721.
- [228] S. Liu, T.L. Yahr, D.W. Frank, J.T. Barbieri, Biochemical relationships between the 53-kilodalton (ExoS3) and 49-kilodalton (ExoS) forms of exoenzyme S of *Pseudomonas aeruginosa*, *J. Bacteriol.* 179 (5) (1997) 1609–1613.
- [229] J. Sun, A.W. Maresso, J.J. Kim, J.T. Barbieri, How bacterial ADP-ribosylating toxins recognize substrates, *Nat. Struct. Mol. Biol.* 11 (9) (2004) 868–876.
- [230] N.C. Simon, J.T. Barbieri, Exoenzyme S ADP-ribosylates Rab5 effector sites to uncouple intracellular trafficking, *Infect. Immun.* 82 (1) (2014) 21–28.
- [231] K. Kawaguchi, S. Yoshida, R. Hatano, S. Asano, Pathophysiological roles of ezrin/radixin/moesin proteins, *Biol. Pharm. Bull.* 40 (4) (2017) 381–390.
- [232] A.W. Maresso, M.R. Baldwin, J.T. Barbieri, Ezrin/radixin/moesin proteins are high affinity targets for ADP-ribosylation by *Pseudomonas aeruginosa* ExoS, *J. Biol. Chem.* 279 (37) (2004) 38402–38408.
- [233] A.W. Maresso, Q. Deng, M.S. Pereckas, B.T. Wakim, J.T. Barbieri, *Pseudomonas aeruginosa* ExoS ADP-ribosyltransferase inhibits ERM phosphorylation, *Cell. Microbiol.* 9 (1) (2007) 97–105.
- [234] A.K. Ganesan, T.S. Vincent, J.C. Olson, J.T. Barbieri, *Pseudomonas aeruginosa* exoenzyme S disrupts Ras-mediated signal transduction by inhibiting guanine nucleotide exchange factor-catalyzed nucleotide exchange, *J. Biol. Chem.* 274 (31) (1999) 21823–21829.
- [235] J. Sun, J.T. Barbieri, *Pseudomonas aeruginosa* ExoT ADP-ribosylates CT10 regulator of kinase (Crk) proteins, *J. Biol. Chem.* 278 (35) (2003) 32794–32800.
- [236] Q. Deng, J. Sun, J.T. Barbieri, Uncoupling Crk signal transduction by *Pseudomonas* exoenzyme T, *J. Biol. Chem.* 280 (43) (2005) 35953–35960.
- [237] B.H. Iglewski, P.V. Liu, D. Kabat, Mechanism of action of *Pseudomonas aeruginosa* exotoxin A: adenosine diphosphate-ribosylation of mammalian elongation factor 2 in vitro and in vivo, *Infect. Immun.* 15 (1) (1977) 138–144.
- [238] R. Jorgensen, A.E. Purdy, R.J. Fieldhouse, M.S. Kimber, D.H. Bartlett, A.R. Merrill, Cholix toxin, a novel ADP-ribosylating factor from *Vibrio cholerae*, *J. Biol. Chem.* 283 (16) (2008) 10671–10678.
- [239] R.J. Fieldhouse, A.R. Merrill, Needle in the haystack: structure-based toxin discovery, *Trends Biochem. Sci.* 33 (11) (2008) 546–556.
- [240] L. Xu, Z.Q. Luo, Cell biology of infection by *Legionella pneumophila*, *Microbes Infect.* 15 (2) (2013) 157–167.
- [241] R.K. Sherwood, C.R. Roy, Autophagy evasion and endoplasmic reticulum subversion: the yin and yang of legionella intracellular infection, *Annu. Rev. Microbiol.* 70 (2016) 413–433.
- [242] B.A. Cunha, A. Burillo, E. Bouza, Legionnaires' disease, *Lancet* 387 (10016) (2016) 376–385.
- [243] H. Ashida, M. Kim, C. Sasakawa, Exploitation of the host ubiquitin system by human bacterial pathogens, *Nat. Rev. Microbiol.* 12 (6) (2014) 399–413.
- [244] J. Qiu, M.J. Sheedlo, K. Yu, Y. Tan, E.S. Nakayasu, C. Das, X. Liu, Z.Q. Luo, Ubiquitination independent of E1 and E2 enzymes by bacterial effectors, *Nature* 533 (7601) (2016) 120–124.
- [245] S. Bhogaraju, I. Dikic, Cell biology: Ubiquitination without E1 and E2 enzymes, *Nature* 533 (7601) (2016) 43–44.
- [246] J.C. Havery, C.R. Roy, Toxicity and sidJ-mediated suppression of toxicity require distinct regions in the sidE family of legionella pneumophila effectors, *Infect. Immun.* 83 (9) (2015) 3506–3514.
- [247] A. Akturk, D.J. Wasilko, X. Wu, Y. Liu, Y. Zhang, J. Qiu, Z.Q. Luo, K.H. Reiter, P.S. Brzovic, R.E. Klevit, Y. Mao, Mechanism of phosphoribosyl-ubiquitination mediated by a single Legionella effector, *Nature* 557 (7707) (2018) 729–733.
- [248] S. Kalayil, S. Bhogaraju, F. Bonn, D. Shin, Y. Liu, N. Gan, J. Basquin, P. Grumati, Z.Q. Luo, I. Dikic, Insights into catalysis and function of phosphoribosyl-linked serine ubiquitination, *Nature* 557 (7707) (2018) 734–738.
- [249] L. Palazzo, B. Thomas, A.S. Jemth, T. Colby, O. Leidecker, K.L. Feijs, R. Zaja, O. Loseva, J.C. Puigvert, I. Matic, T. Helleday, I. Ahel, Processing of protein ADP-ribosylation by Nudix hydrolases, *Biochem. J.* 468 (2) (2015) 293–301.
- [250] C.M. Daniels, P. Thirawatananond, S.E. Ong, S.B. Gabelli, A.K. Leung, Nudix hydrolases degrade protein-conjugated ADP-ribose, *Sci. Rep.* 5 (2015) 18271.
- [251] L. Palazzo, C.M. Daniels, J.E. Nettleship, N. Rahman, R.L. McPherson, S.E. Ong, K. Kato, O. Nureki, A.K. Leung, I. Ahel, ENPP1 processes protein ADP-ribosylation in vitro, *FEBS J.* 283 (18) (2016) 3371–3388.
- [252] K.M. Kotewicz, V. Ramabhadran, N. Sjoblom, J.P. Vogel, E. Haenssler, M. Zhang, J. Behringer, R.A. Scheck, R.R. Isberg, A single legionella effector catalyzes a multistep ubiquitination pathway to rearrange tubular endoplasmic reticulum for replication, *Cell Host Microb.* 21 (2) (2017) 169–181.
- [253] J. Qiu, K. Yu, X. Fei, Y. Liu, E.S. Nakayasu, P.D. Piehowski, J.B. Shaw, K. Puvar, C. Das, X. Liu, Z.Q. Luo, A unique deubiquitinase that deconjugates phosphoribosyl-linked protein ubiquitination, *Cell Res.* 27 (7) (2017) 865–881.
- [254] K.C. Jeong, J.A. Sexton, J.P. Vogel, Spatiotemporal regulation of a Legionella

- pneumophila T4SS substrate by the metaeffector SidJ, *PLoS Pathog.* 11 (3) (2015) e1004695.
- [255] J.A. Ronau, M. Hochstrasser, The DUB blade goes snicker-snack: novel ubiquitin cleavage by a Legionella effector protein, *Cell Res.* 27 (7) (2017) 845–846.
- [256] Y. Ma, P.W. Ludden, Role of the dinitrogenase reductase arginine 101 residue in dinitrogenase reductase ADP-ribosyltransferase binding NAD binding, and cleavage, *J. Bacteriol.* 183 (1) (2001) 250–256.
- [257] S. Nordlund, M. Hogbom, ADP-ribosylation, a mechanism regulating nitrogenase activity, *FEBS J.* 280 (15) (2013) 3484–3490.
- [258] L.F. Huergo, G. Chandra, M. Merrick, P(II) signal transduction proteins: nitrogen regulation and beyond, *FEMS Microbiol. Rev.* 37 (2) (2013) 251–283.
- [259] Y. Zhang, R.H. Burris, P.W. Ludden, G.P. Roberts, Regulation of nitrogen fixation in *Azospirillum brasilense*, *FEMS Microbiol. Lett.* 152 (2) (1997) 195–204.
- [260] Y. Wen, E. Behiels, B. Devreese, Toxin-Antitoxin systems: their role in persistence, biofilm formation, and pathogenicity, *Pathog. Dis.* 70 (3) (2014) 240–249.
- [261] M. Prax, R. Bertram, Metabolic aspects of bacterial persisters, *Front. Cell. Infect. Microbiol.* 4 (2014) 148.
- [262] R. Page, W. Peti, Toxin-antitoxin systems in bacterial growth arrest and persistence, *Nat. Chem. Biol.* 12 (4) (2016) 208–214.
- [263] H.R. Ramage, L.E. Connolly, J.S. Cox, Comprehensive functional analysis of *Mycobacterium tuberculosis* toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution, *PLoS Genet.* 5 (12) (2009) e1000767.
- [264] J.P. Norton, M.A. Mulvey, Toxin-Antitoxin systems are important for niche-specific colonization and stress resistance of uropathogenic *Escherichia coli*, *PLoS Pathog.* 8 (10) (2012) e1002954.
- [265] D. Ren, A.N. Walker, D.A. Daines, Toxin-antitoxin loci vapBC-1 and vapXD contribute to survival and virulence in nontypeable *Haemophilus influenzae*, *BMC Microbiol.* 12 (2012) 263.
- [266] M.A. De la Cruz, W. Zhao, C. Farenc, G. Gimenez, D. Raoult, C. Cambillau, J.P. Gorvel, S. Meresse, A toxin-antitoxin module of *Salmonella* promotes virulence in mice, *PLoS Pathog.* 9 (12) (2013) e1003827.
- [267] F.J. Piscotta, P.D. Jeffrey, A.J. Link, ParST is a widespread toxin-antitoxin module that targets nucleotide metabolism, *Proc. Natl. Acad. Sci. U.S.A.* 116 (3) (2019) 826–834.
- [268] S.Y. Ting, D.E. Bosch, S.M. Mangiameli, M.C. Radey, S. Huang, Y.J. Park, K.A. Kelly, S.K. Filip, Y.A. Goo, J.K. Eng, M. Allaire, D. Veasler, P.A. Wiggins, S.B. Peterson, J.D. Mougous, Bifunctional immunity proteins protect bacteria against FtsZ-targeting ADP-ribosylating toxins, *Cell* 175 (5) (2018) 1380–1392 e14.
- [269] M.A. De Matteis, M. Di Girolamo, A. Colanzi, M. Pallas, G. Di Tullio, L.J. McDonald, J. Moss, G. Santini, S. Bannykh, D. Corda, et al., Stimulation of endogenous ADP-ribosylation by brefeldin A, *Proc. Natl. Acad. Sci. U.S.A.* 91 (3) (1994) 1114–1118.
- [270] V.L. Singleton, N. Bohonos, A.J. Ullstrup, Decumbin, a new compound from a species of *Penicillium*, *Nature* 181 (4615) (1958) 1072–1073.
- [271] S. Spano, M.G. Silletta, A. Colanzi, S. Alberti, G. Fiucci, C. Valente, A. Fusella, M. Salmona, A. Mironov, A. Luini, D. Corda, Molecular cloning and functional characterization of brefeldin A-ADP-ribosylated substrate. A novel protein involved in the maintenance of the Golgi structure, *J. Biol. Chem.* 274 (25) (1999) 17705–17710.
- [272] C. Valente, A. Luini, D. Corda, Components of the CtBP1/BARS-dependent fission machinery, *Histochem. Cell Biol.* 140 (4) (2013) 407–421.
- [273] C. Valente, G. Turacchio, S. Mariggio, A. Pagliuso, R. Gaibisso, G. Di Tullio, M. Santoro, F. Formigini, S. Spano, D. Piccini, R.S. Polishchuk, A. Colanzi, A. Luini, D. Corda, A 14-3-3gamma dimer-based scaffold bridges CtBP1-S/BARS to PI(4)KIIb to regulate post-Golgi carrier formation, *Nat. Cell Biol.* 14 (4) (2012) 343–354.
- [274] M. Bonazzi, S. Spano, G. Turacchio, C. Cericola, C. Valente, A. Colanzi, H.S. Kwon, V.W. Hsu, E.V. Polishchuk, R.S. Polishchuk, M. Sallese, T. Pulvirenti, D. Corda, A. Luini, CtBP3/BARS drives membrane fission in dynamin-independent transport pathways, *Nat. Cell Biol.* 7 (6) (2005) 570–580.
- [275] A. Pagliuso, C. Valente, L.L. Giordano, A. Filograna, G. Li, D. Circolo, G. Turacchio, V.M. Marzullo, L. Mandrich, M.A. Zhukovsky, F. Formigini, R.S. Polishchuk, D. Corda, A. Luini, Golgi membrane fission requires the CtBP1-S/BARS-induced activation of lysophosphatidic acid acyltransferase delta, *Nat. Commun.* 7 (2016) 12148.
- [276] P. Liberali, E. Kakkonen, G. Turacchio, C. Valente, A. Spaar, G. Perinetti, R.A. Bockmann, D. Corda, A. Colanzi, V. Marjomaki, A. Luini, The closure of Pak1-dependent macropinosomes requires the phosphorylation of CtBP1/BARS, *EMBO J.* 27 (7) (2008) 970–981.
- [277] J.S. Yang, H. Gad, S.Y. Lee, A. Mironov, L. Zhang, G.V. Beznoussenko, C. Valente, G. Turacchio, A.N. Bonsra, G. Du, G. Baldanzi, A. Graziani, S. Bourgoin, M.A. Frohman, A. Luini, V.W. Hsu, A role for phosphatidic acid in COPI vesicle fission yields insights into Golgi maintenance, *Nat. Cell Biol.* 10 (10) (2008) 1146–1153.
- [278] A. Colanzi, C. Hidalgo Carcedo, A. Persico, C. Cericola, G. Turacchio, M. Bonazzi, A. Luini, D. Corda, The Golgi mitotic checkpoint is controlled by BARS-dependent fission of the Golgi ribbon into separate stacks in G2, *EMBO J.* 26 (10) (2007) 2465–2476.
- [279] G. Chinnadurai, The transcriptional corepressor CtBP: a foe of multiple tumor suppressors, *Cancer Res.* 69 (3) (2009) 731–734.
- [280] A. Colanzi, G. Grimaldi, G. Catara, C. Valente, C. Cericola, P. Liberali, M. Ronci, V.S. Lalioti, A. Bruno, A.R. Beccari, A. Urbani, A. De Flora, M. Nardini, M. Bolognesi, A. Luini, D. Corda, Molecular mechanism and functional role of brefeldin A-mediated ADP-ribosylation of CtBP1/BARS, *Proceedings of the National Academy of Sciences of the United States of America*, 2013, pp. 9794–9799.
- [281] M. Nardini, S. Spano, C. Cericola, A. Pesce, A. Massaro, E. Millo, A. Luini, D. Corda, M. Bolognesi, CtBP/BARS: a dual-function protein involved in transcription co-repression and Golgi membrane fission, *EMBO J.* 22 (12) (2003) 3122–3130.
- [282] A. Peyroche, B. Antonny, S. Robineau, J. Acker, J. Cherfils, C.L. Jackson, Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain, *Mol. Cell* 3 (3) (1999) 275–285.
- [283] J.G. Donaldson, D. Finazzi, R.D. Klausner, Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein, *Nature* 360 (6402) (1992) 350–352.
- [284] R.W. Doms, G. Russ, J.W. Yewdell, Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum, *J. Cell Biol.* 109 (1) (1989) 61–72.
- [285] T. Fujiwara, K. Oda, S. Yokota, A. Takatsuki, Y. Ikehara, Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum, *J. Biol. Chem.* 263 (34) (1988) 18545–18552.
- [286] J. Lippincott-Schwartz, L.C. Yuan, J.S. Bonifacio, R.D. Klausner, Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER, *Cell* 56 (5) (1989) 801–813.
- [287] J. Lippincott-Schwartz, L. Yuan, C. Tipper, M. Amherdt, L. Orci, R.D. Klausner, Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic, *Cell* 67 (3) (1991) 601–616.
- [288] G. Tamura, K. Ando, S. Suzuki, A. Takatsuki, K. Arima, Antiviral activity of brefeldin A and verrucarin A, *J. Antibiotics* 21 (2) (1968) 160–161.
- [289] M.J. Crabbe, V. Betina, Structural correlates of antifungal and cytotoxic activity of brefeldin A: the importance of a rigid planar lactone ring, *Folia Microbiol.* 39 (5) (1994) 445–448.
- [290] V. Betina, Effects of the macrocyclic antibiotic cyanine on HeLa cells growth and metabolism, *Neoplasma* 16 (1) (1969) 23–32.
- [291] D.E. Larsson, M. Wickstrom, S. Hassan, K. Oberg, D. Granberg, The cytotoxic agents NSC-95397, brefeldin A, bortezomib and sanguinarine induce apoptosis in neuroendocrine tumors in vitro, *Anticancer Res.* 30 (1) (2010) 149–156.
- [292] J.L. Moon, S.Y. Kim, S.W. Shin, J.W. Park, Regulation of brefeldin A-induced ER stress and apoptosis by mitochondrial NADP(+) dependent isocitrate dehydrogenase, *Biochem. Biophys. Res. Commun.* 417 (2) (2012) 760–764.
- [293] M.E. Ivarsson, J.C. Leroux, B. Castagner, Targeting bacterial toxins, *Angew. Chem. Int. Ed. Engl.* 51 (17) (2012) 4024–4045.
- [294] Z. Turgeon, R. Jorgensen, D. Visschedyk, P.R. Edwards, S. Legree, C. McGregor, R.J. Fieldhouse, D. Mangroo, M. Schapira, A.R. Merrill, Newly discovered and characterized antiviral compounds inhibit bacterial mono-ADP-ribosyltransferase toxins, *Antimicrob. Agents Chemother.* 55 (3) (2011) 983–991.
- [295] P. Cherubin, M.C. Garcia, D. Curtis, C.B. Britt, J.W. Craft Jr., H. Burress, C. Berndt, S. Reddy, J. Guyette, T. Zheng, Q. Huo, B. Quinones, J.M. Briggs, K. Teter, Inhibition of cholera toxin and other AB toxins by polyphenolic compounds, *PLoS One* 11 (11) (2016) e0166477.
- [296] M. Saleeb, C. Sundin, O. Aglar, A.F. Pinto, M. Ebrahimi, A. Forsberg, H. Schuler, M. Eloffson, Structure-activity relationships for inhibitors of *Pseudomonas aeruginosa* exoenzyme S ADP-ribosyltransferase activity, *Eur. J. Med. Chem.* 143 (2018) 568–576.
- [297] M. Rownicki, T. Pienko, J. Czarnecki, M. Kolanowska, D. Bartosik, J. Trylska, Artificial activation of *Escherichia coli* mazEF and hipBA toxin-antitoxin systems by antisense peptide nucleic acids as an antibacterial strategy, *Front. Microbiol.* 9 (2018) 2870.
- [298] V. Khodaverdian, M. Pesho, B. Truitt, L. Bollinger, P. Patel, S. Nithianantham, G. Yu, E. Delaney, E. Jankowsky, M. Shoham, Discovery of antiviral agents against methicillin-resistant *Staphylococcus aureus*, *Antimicrob. Agents Chemother.* 57 (8) (2013) 3645–3652.
- [299] V.W. Soo, B.W. Kwan, H. Quezada, I. Castillo-Juarez, B. Perez-Eretza, S.J. Garcia-Contreras, M. Martinez-Vazquez, T.K. Wood, R. Garcia-Contreras, Repurposing of anticancer drugs for the treatment of bacterial infections, *Curr. Top. Med. Chem.* 17 (10) (2017) 1157–1176.
- [300] S. Muhlen, P. Dersch, Anti-virulence strategies to target bacterial infections, *Curr. Top. Microbiol. Immunol.* 398 (2016) 147–183.