New players to the field of ADP-ribosylation make the final cut

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ADP-ribose-based intermediates, including PARP-generated mono- and poly(ADP-ribose) post-translational modifications, are important to a number of cellular signalling processes. The reversal of poly(ADP-ribosylation) is mostly attributed to PARG, which however cannot remove the final protein-linked mono(ADP-ribose) residue. Three recent studies, one of them in *The EMBO Journal*, now report that certain macrodomains remove terminal ADP-ribose modifications from acidic residues.

ADP-ribosylation is a post-translational modification (PTM) in which the ADP-ribose moiety of NAD⁺ is covalently transferred to a target protein (see Figure 1). This

reaction is catalysed by poly(ADP-ribose) polymerase (PARP) enzymes, some of which also extend the modification by adding additional ADP-ribose molecules through ribose-ribose bonds, thus generating poly(ADP-ribose) (PAR). PARP1 and PARP2 account for the majority of PAR formation in response to cellular stress such as DNA damage.

Poly(ADP-ribosylation) is a dynamic and transient modification event, since an enzyme called poly(ADP-ribose) glycohydrolase (PARG) counters PARP activities by degrading PAR through endo- and exo-glycosidic activities (see Figure 1). However, neither PARG nor the unrelated ADP-ribosyl hydrolase 3 (ARH-3) also implicated in PAR hydro-

Figure 1 Pathway of reversible protein modification reactions that involve NAD⁺ consumption. Consumption of NAD⁺ through nicotinamide cleavage drives the catalysis of mono- and poly(ADP-ribosylation) of proteins by ADP-ribosyl transferases, as well as deacetylation of proteins by Sirtuins. Formation of these modifications is important to various cell signalling events such as DNA repair, chromatin remodelling, transcription, telomere homeostasis, and cell death. ADP-ribose modifications are short-lived due to the activity of hydrolase enzymes reversing the modification to yield ADP-ribose. The recently identified macrodomains C6orf130/TARG, MacroD1, and MacroD2 now fill in previously unidentified roles of ADP-ribose and PAR hydrolysis from acidic residues.

lysis, are capable of removing the terminal ADP-ribose residue from proteins, leaving them mono-(ADP-ribosyl)ated (Moss *et al*, 1992; Slade *et al*, 2011). Lack of knowledge about the enzymes that remove mono-ADP-ribose modifications has left the complete reversibility of this dynamic cycle unclear.

Bridging this gap in understanding of the ADP-ribosylation cycle, three separate studies have now linked macrodomains to mono-(ADP-ribose)hydrolase activities (Jankevicius *et al*, 2013; Rosenthal *et al*, 2013; Sharifi *et al*, 2013). The ubiquitous and widely conserved macrodomain is typically found as part of larger proteins, and its importance to the field of ADP-ribosylation stems from the ability to recognize various products of NAD-consuming reactions, such as ADP-ribosylated proteins, PAR, ADP-ribose, ADP-ribose-1'' phosphate, and O-acetyl-ADP-ribose (Karras *et al*, 2005). Some macrodomains have also been found to be catalytically active, exhibiting O-acetyl-ADP-ribose deacetylase and ADP-ribose phosphatase activity (Karras *et al*, 2005; Chen *et al*, 2011; Peterson *et al*, 2011). Still, several other macrodomains neither possess catalytic activity nor bind products of NAD⁺ metabolism, and remain classified with unknown ligand binding status.

In this issue of *The EMBO Journal*, Sharifi *et al* (2013) identify the macrodomain-containing protein C6orf130/TARG as capable of interacting with PARP and removing mono(ADP-ribose) from it. Writing in *Nature Structure and Molecular Biology*, Rosenthal *et al* (2013) and Jankevicius *et al* (2013) additionally report MacroD1 and MacroD2 macrodomains as capable of reversing PARP-mediated ADP-ribosylation. These three human macrodomains are all efficient at hydrolysing the ribose-acceptor bond only when the acceptor residue is either an aspartic or glutamic acid, which are the predominant modification sites identified in the major cellular PAR acceptor, PARP1 (Chapman *et al*, 2013). On the other hand, unlike PARG or ARH-3, these macrodomains are unable to cleave the ribose-ribose bonds within the PAR polymer. Interestingly, Sharifi *et al* found C6orf130/TARG to nevertheless bind PAR and cleave the ribose-acceptor bond of PAR-modified PARP1, removing the entire chain *en bloc*. Such activity could have important implications for the production of free poly(ADP-ribose) chains, which may also serve as signalling molecules (Andrabi *et al*, 2006).

Although C6orf130/TARG lacks extended N- and C-terminal structural features present in other macrodomain proteins (Peterson *et al*, 2011), X-ray crystallography and structural modelling shows that all three macrodomains align well with regard to their structural cores (Jankevicius *et al*, 2013; Rosenthal *et al*, 2013; Sharifi *et al*, 2013). Nonetheless, each study presents a distinct perspective on the proposed mechanism of action. Rosenthal *et al* suggest a mechanism related to MacroD1's deacetylase mechanism (Chen *et al*, 2011), in which a conserved aspartic acid residue acts as a base to deprotonate a nearby coordinated water molecule, thus facilitating hydrolysis of the ester bond. However, mutation of this residue only reduces ADP-ribose hydrolysis activity, indicating that this catalytic acidic residue may not be an exclusive actor in deprotonation. Jankevicius *et al* (2013) define a macrodomain signature motif central to catalytic activity, and propose a substrate-assisted mechanism in which the ADP-ribose α -phosphate activates the coordinated water molecule for nucleophilic attack on

the carbonyl carbon. Irrespective of water activation mechanism, both reports agree that steric disruptions and displacement of the coordinated water molecule in the active site abolish activity. For C6orf130/TARG, Sharifi *et al* (2013) make the unique observation that mutating the catalytic aspartic acid residue into alanine results in the enzyme becoming cross-linked to the ADP-ribosylated protein. This leads them to propose that a catalytic lysine residue first forms a covalent intermediate with the ribose ring through an Amadori rearrangement mechanism, followed by hydrolysis mediated by the catalytic aspartic acid residue. C6orf130/TARG does not share the catalytic residue signature of MacroD1 and MacroD2, correlating with the proposed mechanistic differences, but all three can essentially be defined as ADP-ribose 1'' ester hydrolases.

Rosenthal *et al* (2013) show that MacroD2 is capable of completely removing ADP-ribose from PARP10, which is only modified through glutamic acid residues, but not completely from histone H1 or PARP1, in line with reports of alternative lysine acceptor sites on those targets (Altmeyer *et al*, 2009; Messner *et al*, 2010). Discrimination for distinct residues is not unusual for enzymes that catalyse ADP-ribosyl hydrolysis: ARH-1, for example, is known to remove ADP-ribose from arginine, but not from glutamate or lysine residues (Moss *et al*, 1992). Residue preference is likely attributed to the chemical nature of the side-chain group, but selectivity may also be influenced by hydrolase-target interactions, for example by the structure and sequence of residues neighbouring the ADP-ribose-modified glutamate. Still, room remains for putative enzymes that can remove ADP-ribose from lysine residues, and other ADP-ribose binding modules aside from macrodomains could be endowed with additional catalytic properties. It is therefore tempting to speculate that the family of ADP-ribose hydrolases is not yet complete.

Our understanding of the biology of mono(ADP-ribose) transferases (mARTs) is just developing. The identification of enzymes that remove the terminal ADP-ribose from modified proteins help establish the mono(ADP-ribosyl)ation cycle, and will allow us to advance our understanding in new ways. These new players also raise important questions regarding poly(ADP-ribosyl)ation. For instance, a precise coordination between the activities of PARP1 and PARG in response to genotoxic stress is critical to repair damaged DNA and maintain genomic integrity (Gao *et al*, 2007). Is the removal of mono(ADP-ribose) from PARP1 at specific sites essential for the reactivation of PARP1 during cycling of PAR synthesis and degradation? While MacroD1 is predominantly localized to the mitochondria, MacroD2 and C6orf130/TARG show nuclear localization and PARP-dependent accumulation at DNA damage sites (Jankevicius *et al*, 2013; Sharifi *et al*, 2013). Recruitment of MacroD2 to sites of damage is biphasic, indicating likely coordination with the activities of PARP1 and PARG. This recruitment may imply a protective role by suppressing PARP1 activity under normal conditions, since PARP1 overactivation or the absence of PARG can lead to excessive, cytotoxic amounts of PAR (Andrabi *et al*, 2006).

PARPs have received much attention in the past decade because of PARP inhibitors that selectively target DNA repair-deficient cancers. With the major advances in understanding the enzymes that reverse this process, an evaluation of

therapeutic potential through inhibition of PAR hydrolysis will be significant. There are currently no established ADP-ribose hydrolase inhibitors (aside from ADP-HPD), and PARG inhibitors are not yet suitable for cell-based testing. These new macrodomain structures as well as others solved in recent years, including PARG (Slade *et al*, 2011), leave the

field of ADP-ribosylation rich in opportunity for structure-guided drug design approaches.

Conflict of interest

The authors declare that they have no conflict of interest.

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