Trends in
Biochemical Sciences

Spotlight

Modifying the modifiers: ubiquitination of ADP-ribosylation in human cells

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Ubiquitination and ADP-ribosylation are protein post-translational modifications (PTMs) which influence diverse protein properties. *In vitro* work has indicated that ubiquitin can be ADP-ribosylated and vice versa, ADP-ribose ubiquitinated. An exciting new study by Bejan *et al*. now demonstrates that ubiquitination of ADP-ribosylated proteins, termed MARUbylation, occurs in human cells.

PTMs of proteins allow rapid functional changes to the cellular proteome, as every modification has the potential to regulate specific protein properties such as activity, localisation, or stability. PTMs thus enable cells to respond rapidly to a wide range of stimuli. Ubiquitination is a PTM where the small protein ubiquitin is attached to substrates via a concerted action of an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ligase [1]. Chains of polyubiquitin can be generated via one of seven lysines present in ubiquitin and at the N terminus. Polyubiquitination via lysine 48 (K48) can lead to degradation, whereas other linkages, including K63-linked polyubiquitin, have signalling functions. In contrast to ubiquitination, ADP-ribosylation is a PTM which remains more enigmatic, especially mono(ADP-ribosyl)ation. In a reaction consuming NAD^+, the 17 members of the so-called PARP family transfer ADP-ribose (ADPr) while releasing nicotinamide. Initially, these enzymes were thought to function as polymerases, hence the name poly(ADP-ribosyl)polymerase or PARP. We now know that most of the ‘PARPs’ transfer only a single ADPr or generate poly(ADPr) by iterative ADPr transfer, with none of the family members having polymerase activity. Transferase activity notwithstanding, the name PARP remains [2]. PTMs can be reversed by diverse enzymes sometimes collectively referred to as ‘erasers’, with enzymes generating PTMs termed ‘writers’.

Interaction between ubiquitination and ADP-ribosylation has been observed: PARP10 contains ubiquitin interacting motifs which bind polyubiquitin, whereas several ubiquitin E3 ligases harbour poly(ADPr) binding domains to allow binding to and subsequent ubiquitination of poly(ADP-ribosyl)ated proteins. An intriguing crosstalk between these PTMs was surprisingly found in prokaryotes, which do not have a ubiquitination system. The effector protein SdeA of *Legionella pneumophila*, which is secreted into the host cytosol, has ADP-ribosyltransferase and phosphodiesterase activity [3]. This unique combination of activities allows it to generate a phosphoribosylated form of host ubiquitin, modified on arginine 42, which results in serine ubiquitination while impairing canonical ubiquitination. Recent *in vitro* experiments took the ubiquitin-ADPr crosstalk, also in mammals, a step further: the modifications can not only be added consecutively, but they can also be transferred onto each other. First, it was shown that ubiquitin is ADP-ribosylated by a PARP9/DTX3 E3 ligase dimer [4]; however, this was later shown to occur independent of PARP9, leaving the exact role of PARP9 unclear [5]. Vice versa, DTX E3 ligases can ubiquitinate ADPr *in vitro* [6]. The question which remained unanswered was whether this represents a physiological modification.

The current work from Bejan *et al*. explores exactly this question [7]. When examining lysates from cells overexpressing GFP-PARP10, the authors observed that the ADP-ribosylated form of PARP10 appears not in the form of a uniformly migrating protein species on western blot, as would be expected for mono(ADP-ribosyl)ation, but instead presents as smear. Incubation with a promiscuous deubiquitinating enzyme (DUB) led to its collapse into a distinct band, indicating that ADPr-PARP10 is also polyubiquitinated. In canonical ubiquitination, ubiquitin is attached to lysine by an isopeptide linkage, whereas non-canonical ubiquitination on serine or threonine occurs via an ester (Table 1). The authors exploited the different chemical nature of these linkages to explore the modification present, as canonical ubiquitination is not affected by neutral hydroxylamine (NH_2OH), but ubiquitin-serine and ADPr-glutamate are highly sensitive. Incubation with NH_2OH led to a reduction of the observed PARP10 smear, indicating that ubiquitin is attached via ADPr or is present on serine. The authors further probed the nature of the ubiquitin linkage using a bacterial DUB, TssM, which in mutated form, TssM*, loses isopeptidase activity but functions as a highly efficient esterase. As the PARP10 modification can be reversed by TssM*, the authors concluded that an ester-linked ubiquitin is present. Incubation of immunoprecipitated PARP10 with NUDIX16, a phosphodiesterase which releases AMP from the ADPr, led to release of polyubiquitin. This experiment provides strong evidence that PARP10 is modified with polyubiquitin directly attached to ADPr. The released polyubiquitin can be processed by the K11-specific DUB Cezanne, showing that the ADPr-linked polyubiquitin contains K11-polyubiquitin. Lastly, the authors incubated cells with interferon beta to elevate endogenous PARP10 and PARP14 levels. Incubation of the lysates with TssM* led to a compaction of the smeared signal to a distinct band, hinting at the presence of the dual modification on endogenous proteins. Although only a minor percentage of cellular PARP10 is modified with ubiquitin linked to ADPr, this innovative study provides the first evidence that it exists. The authors

Table 1. Overview of the enzymes involved in MARUbylation

Modification	Transferase	Linkage	Erasers	Structure (pink line indicating eraser activity)	Sensitive to NH_2OH
Poly(ADP-ribosyl)ation	PARP1 & PARP2, TNKS1 & TNKS2	*O*-glycosidic	PARG, ARH3		No
Mono(ADP-ribosyl)ation	PARP3 & PARP4, PARP6-12, PARP14-16	Ester[a]	Macrodomain-containing proteins		Yes
Ubiquitination	Many ubiquitin ligases	Isopeptide (lysine)	Deubiquitinating enzymes	Canonical	No
		Ester (serine or threonine)		Non-canonical R = H (serine) R = CH_3 (threonine)	Yes
MARUbylation	DTX E3 ligases	Ester	Macrodomain -containing proteins, NUDIX[b], DUBs[b], as yet unknown erasers?		Yes

[a]An ester bond is present if ADP-ribose is attached to a glutamate or aspartate. ADP-ribose can be attached to other amino acids such as serine or cysteine via different linkages, which is not displayed here.

[b]Processing of MARUbylation by NUDIX or DUB enzymes will leave residual phosphoribose or ADP-ribose, respectively.

have suggested naming this dual modification MARUbylation.

The exciting finding that MARUbylation exists raises many questions. How does MARUbylation regulate the modified PARPs? PARP10 and PARP14 localise to dynamic, ubiquitin-rich foci [8], and it would be intriguing to incubate microscopy samples with NH_2OH or NUDIX16; presumably, this would lead to a loss of ADPr in said foci, but will it also lead to a reduction of ubiquitin staining? Both PARP10 and PARP14 are also involved in the antiviral response via poorly understood mechanisms [9], making it tempting to speculate about a role of MARUbylation therein. Furthermore, the current paper does not yet explore the breadth of this modification. Is it limited to ubiquitination of PARP automodification or is MARUbylation more widespread? DTX E3 ligases can add ubiquitin onto free ADPr or ADPr-peptides *in vitro* [6], but can they add ubiquitin to any ADP-ribosylated proteins in cells or do they recognise specific motifs? Depending on the amino acid which is

ADP-ribosylated, ADP-ribosylation can be sensitive to both temperature and pH [10], and any ubiquitinated ADPr present may have gone unnoticed if samples were prepared in unfavourable conditions. The modification may thus be more prevalent than we currently know. To fully probe the extent of MARUbylation in cells, it will be essential to develop specific detection reagents, as well as to consider the possibility of erasers specific for the dual modification. Bejan *et al*.'s study of MARUbylation in cells paves the way for in-depth research on its function, which we undoubtedly will hear more about in time to come.

Declaration of interests

The authors declare no competing interests.

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