Molecular Cell

Supplemental Information

The Promise of Proteomics for the Study of ADP-Ribosylation

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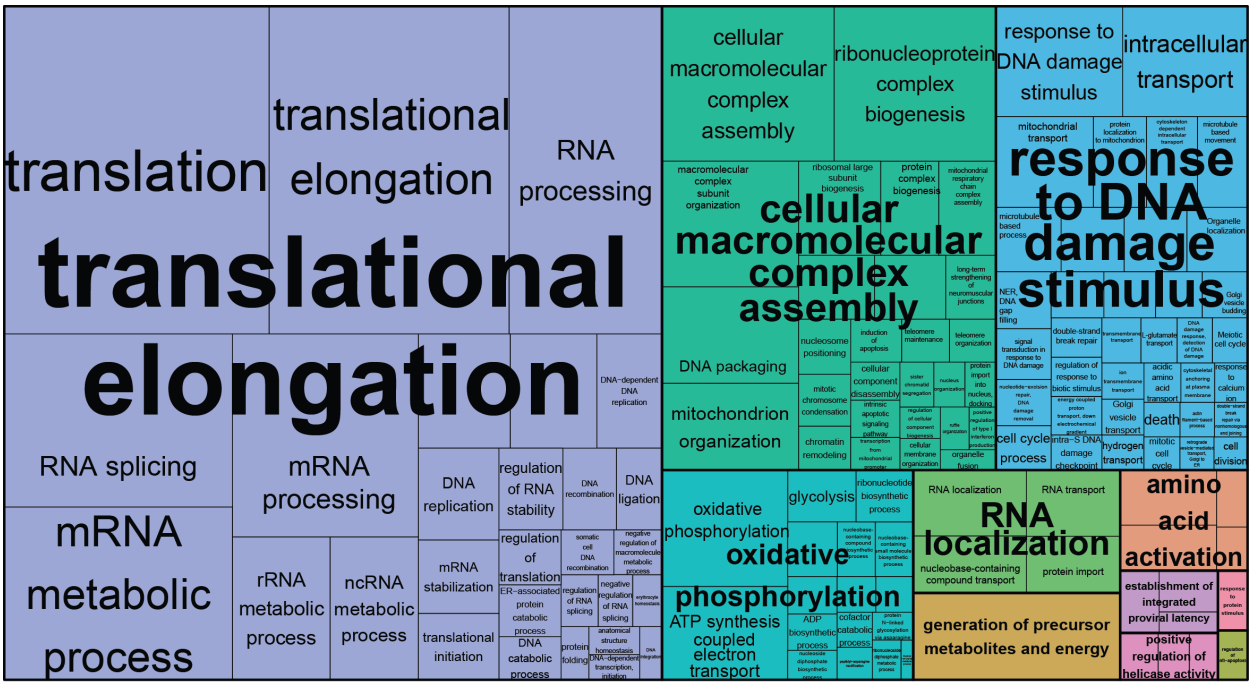

Figure S1. Processes enriched in the ADP-ribosylated interactome (full table of Figure 2B). Gene ontology determined using DAVID, treemap constructed using REViGO and R. Source data available in Table S2.

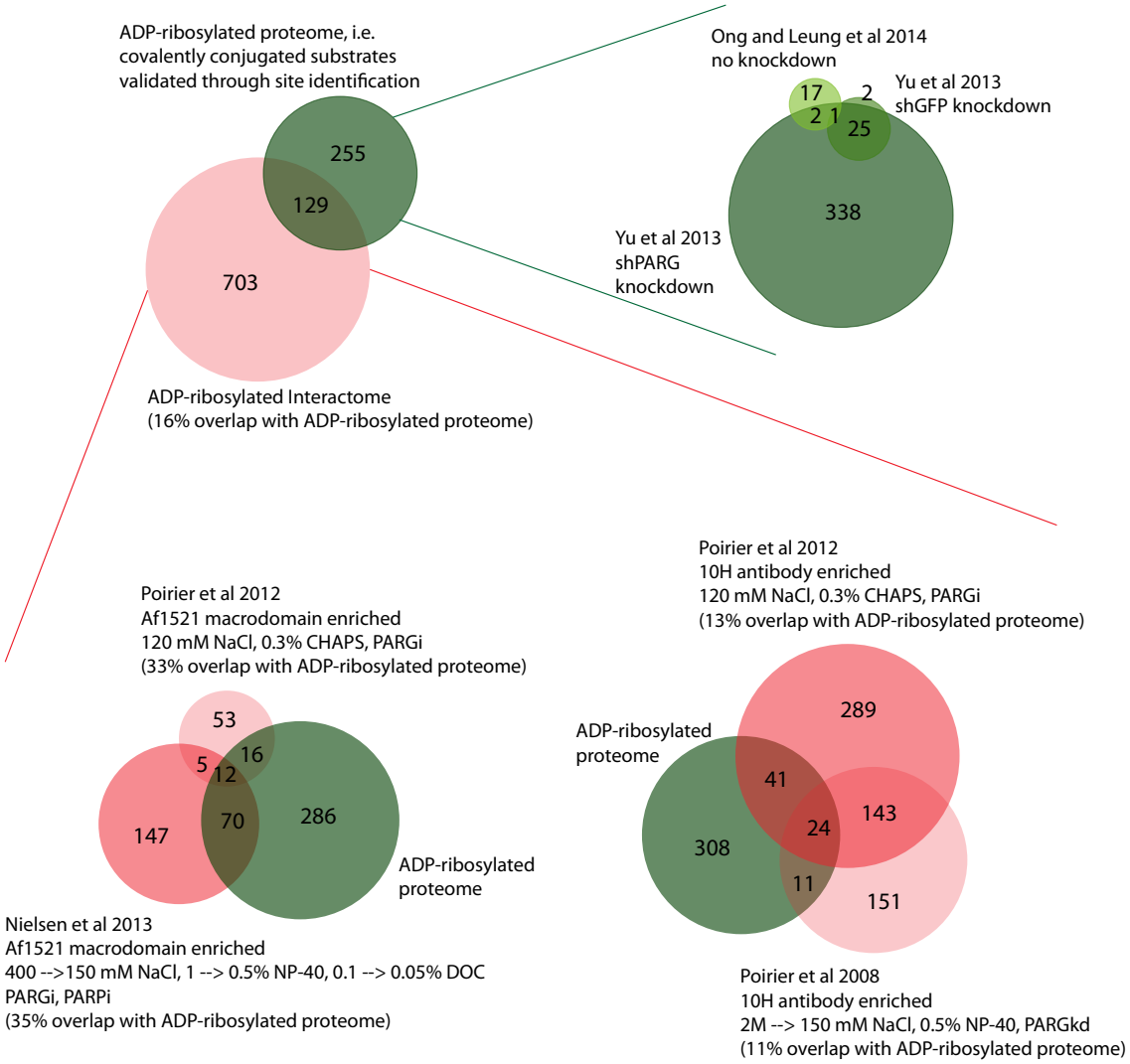

Figure S2. Comparing the ADP-ribosylated interactomes with the known ADP-ribosylated proteomes. PARGi, PARG inhibitor; PARPi, PARP inhibitor; PARGkd, PARG knockdown. Interactome studies, including protein extraction and enrichment conditions, are described in detail in Figure 2A. Source data available in Table S2.

Table S1. Functions attributed to PARP family members
Supporting examples from the literature for the connection made between each PARP and each cellular process in Figure 1.

PARP	Genome Maintenance	Transcription	Translation and mRNA stability	Cell cycle	Cell death	Cell structure and motility	Transport	Metabolism	Cell signaling
1	(de Murcia et al., 1997)	(Kraus and Hottiger, 2013)		(Saxena et al., 2002)	(Hong et al., 2004)			(Bai and Canto, 2012)	(Andreone et al., 2003)
2	(Ame et al., 1999)	(Szanto et al., 2012)		(Liang et al., 2013)	(Li et al., 2010)			(Bai and Canto, 2012)	(Wyrsch et al., 2012)
3	(Boehler et al., 2011)	(Rouleau et al., 2011)		(Augustin et al., 2003)					
4				(Kickhoefer et al., 1999)			(van Zon et al., 2003)		
5a	(Smith et al., 1998)		(Leung et al., 2011)	(Chang et al., 2005)	(Bae et al., 2003)		(Yeh et al., 2007)		(Huang et al., 2009)
5b	(Cook et al., 2002)				(Kaminker et al., 2001)				(Huang et al., 2009)
6				(Tuncel et al., 2012)					
7		(MacPherson et al., 2013)		(Roper et al., 2014)					
8									
9	(Yan et al., 2013)	(Aguiar et al., 2005)							(Juszczynski et al., 2006)
10	(Nicolae et al., 2014)	(Yu et al., 2005)		(Chou et al., 2006)	(Herzog et al., 2013)				(Verheugd et al., 2013)
11						(Meyer-Ficca et al., 2015)			
12			(Welsby et al., 2014)						(Atasheva et al., 2014)
13			(Leung et al., 2011)						(Hayakawa et al., 2011)
14	(Nicolae et al., 2015)	(Mehrotra et al., 2011)	(Iqbal et al., 2014)			(Vyas et al., 2013)		(Cho et al., 2011)	(Goenka and Boothby, 2006)
15		(Aguiar et al., 2005)	(Leung et al., 2011)						
16							(Di Paola et al., 2012)		(Jwa and Chang, 2012)

References for supporting examples of PARP functions:

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Table S2. The ADP-ribose interactome
Source data used to generate the ADP-ribosylated interactomes shown in Figure 2. See Supplementary Text for criteria of data inclusion.

Table S3. PARP specific substrates
Source data used to generate the Euler diagrams showing PARP-1, PARP-2, PARP-10 and PARP-14 specific substrates in Figure 5.

Table S4. Auto-modification sites identified on PARP-1
Source data used to generate the PARP-1 auto-modification site map from Figure 6.

A cautionary note—the possibility of non-enzymatic ADP-ribosylation

Non-enzymatic modification of amino acids by ADP-ribose has been identified by several pioneers in the field, including studies by Drs. Elaine and Myron Jacobson (e.g. Cervantes-Laurean et al., 1996), Joel Moss (e.g. McDonald and Moss, 1994), and mentioned in reviews by Drs. Alexander Bürkle (Bürkle, 2005), Guy Poirier (D'Amours et al., 1999) and Michael Hottiger (Hassa et al., 2006). These original studies clearly indicated the possibility for ADP-ribose to non-enzymatically conjugate to lysine, arginine and cysteine residues in vitro, using different analytical tools, including NMR, chromatography profile, and sensitivities to different chemicals. As stated in Cervantes-Laurean et al., 1996, the chemical conjugation of ADP-ribose (also known as glycation) was added to proteins in a concentration-dependent manner and can occur even within 8 minutes (the shortest timepoint sampled). Figure 5 in this 1996 paper further illustrated different rates of chemical ADP-ribosylation for different histones that have different numbers of lysines (e.g. H1 with 56 lysines incorporated less than H4 with 11 lysines), suggesting that the incorporation may not be random. Drawing a parallel to non-enzymatic glycation by glucose, only specific lysine residues in albumin are chemically conjugated in vivo (Iberg and Flückiger, 1986), therefore it is not clear whether each lysine is equally non-enzymatically modified.

Given the highly sensitive nature of state-of-the-art mass spectrometry, the stable nature of ADP-ribose-lysine/arginine bond and the use of enrichment tools to identify ADP-ribosylated sites, it is experimentally feasible to detect even low level of artificial “noise” from biological samples prepared for proteomics studies. We would like to note that ADP-ribose can be generated by (1) PARG/ARH3 conversion of PARylation sites to MARylation sites for site identification and/or potentially *in vivo* (Messner et al., 2010; Rosenthal et al., 2011), or (2) PARPs when acting as NADases (e.g., Desmarais et al., 1991). In addition, ADP-ribose has been used as an elution condition for macrodomain enrichment for ADP-ribosylated proteins (Dani et al., 2009), which would not be a problem in identifying substrates; however, the excessive amount of free ADP-ribose used for elution could potentially chemically conjugate the substrates in the eluates in vitro, resulting in the identification of non-physiological ADP-ribosylation sites by mass spectrometry. Given that the effect of chemical conjugation by ADP-ribose in proteomic studies remains uncertain, the field would greatly benefit from performing a series of experiments to clearly establish whether or not non-enzymatic ADP-ribosylation should be a concern for proteomics studies.

Criteria for proteomics datasets analyzed in Supplementary Table 2

We have analyzed all four proteomic studies undertaken so far that identify ADP-ribosylated substrates along with their interactors (i.e., the ADP-ribosylated interactome) (Gagné et al., 2008; 2012; Isabelle et al., 2012; Jungmichel et al., 2013). We note that the Jungmichel *et al* 2013 study is aimed at mapping ADP-ribosylated targets (substrates) during DNA damage. However, based on the materials and methods in Jungmichel *et al* (2013), PAR enrichment was performed in cell lysis buffer containing 50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.05% sodium deoxycholate, protease inhibitors, phosphatase inhibitors, PARG inhibitor and PARP inhibitor. This buffer most closely resembles the modified RIPA buffer commonly used in proteomics for protein–protein interactome enrichment (e.g. by the Matthias' Mann group in Ong and Mann, 2006), though contains less of the ionic detergent which represents the harshest denaturant in the buffer (0.05% sodium deoxycholate vs. 0.25% in Ong and Mann, 2006). Standard RIPA buffer, which contains 1% sodium deoxycholate, is also commonly employed for the study of protein-protein interactions (Sefton, 2001). Therefore, conservatively, it can reasonably be assumed that the proteins identified in this study constitute ADP-ribosylated interactome, i.e. ADP-ribosylated proteins, ADP-ribose interactors, and protein–protein interaction partners of both the former and latter. However, we noted that the lysis conditions used are slightly more stringent than those used by Poirier and co-workers in their 2008 and 2012 studies as summarized in Figure 2A (Gagné et al., 2008; 2012; Isabelle et al., 2012). Using the current set of 384 ADP-ribosylated substrates with defined sites mapped (Daniels et al., 2014; Zhang et al., 2013), 82 of these proteins overlap with the Jungmichel 2013 data, accounting for ~35% of their *Af*1521 macrodomain-enriched proteome. On the other hand, there are 28 proteins overlapping with the Gagné 2012 data, accounting for a comparable ~33% of their *Af*1521 macrodomain-enriched proteome (see Figure S2 above). The differences in induction, lysis and enrichment conditions, cell lines as well as limited sampling, in general, could all contribute to the different degrees of overlap. The actual proportion of ADP-ribosylated substrates vs. binding proteins found in these respective studies can be determined with approaches that identify specific sites of ADP-ribosylation in future studies. Given that the condition reported in Jungmichel et al (2013) cannot exclude the inclusion of proteins that binds to ADP-ribosylated substrates, we therefore take a conservative approach and discuss the paper in the context among other pioneering works of ADP-ribosylated interactomes.

In order to control the many variables present in the meta-analysis presented in Figure 2B, the proteins identified through PARG-dead enrichment were omitted, as they represent a single dataset, whereas the 10H (Gagné et al., 2008; 2012; Isabelle et al., 2012) and macrodomain (Gagné et al., 2008; Jungmichel et al., 2013) enriched protein groups represent at least two independent datasets.

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