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REVIEW ARTICLE

Mechanisms governing PARP expression, localization, and activity in cells

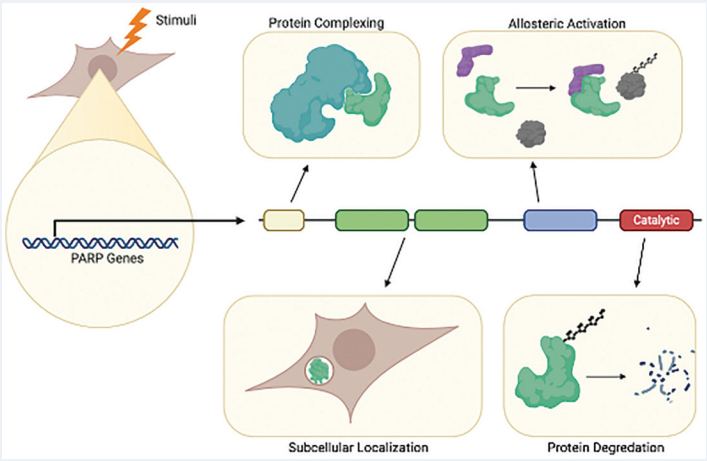
Daniel J. Sanderson and Michael S. Cohen

Department of Chemical Physiology and Biochemistry, Oregon Health and Science University, Portland, OR, USA

ABSTRACT

Poly-(ADP)-ribose polymerases (PARPs) are a family of 17 enzymes in humans that have diverse roles in cell physiology including DNA damage repair, transcription, innate immunity, and regulation of signaling pathways. The modular domain architecture of PARPs gives rise to this functional diversity. PARPs catalyze the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD^+) to targets—proteins and poly-nucleic acids. This enigmatic post-translational modification comes in two varieties: the transfer of a single unit of ADP-ribose, known as mono-ADP-ribosylation (MARylation) or the transfer of multiple units of ADP-ribose, known as poly-ADP-ribosylation (PARylation). Emerging data shows that PARPs are regulated at multiple levels to control when and where PARP-mediated M/PARylation occurs in cells. In this review, we will discuss the latest knowledge regarding the regulation of PARPs in cells: from transcription and protein stability to subcellular localization and modulation of catalytic activity.

GRAPHICAL ABSTRACT



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PARPs comprise a small, yet functional diverse enzyme family

Poly-(ADP)-ribose polymerases (PARPs) are a family of structurally and functionally diverse enzymes (17 in humans, PARPs 1–16) in humans catalyzing the post-translational modification (PTM) termed ADP-ribosylation. PARPs can be divided into two major subfamilies: PARPs (PARP3, 6–12, 14–16) that catalyze the transfer of a single unit of ADP-ribose from nicotinamide adenine dinucleotide (NAD^+), a process known as MARylation; and PARPs (PARP1, 2, 4, 5a, 5b) that catalyze the transfer of multiple units of ADP-ribose from NAD^+, a process known as PARylation (Figure 1(a,b)). One family member, PARP13.1 (long isoform), is catalytically inactive (Figure 1(b)) (Vyas et al. 2014); the other major PARP13 isoform, PARP13.2, is missing the catalytic domain altogether. M/PARylation occurs on a variety of nucleophilic amino acids in protein targets—including glutamate, aspartate, serine, tyrosine, cysteine, lysine, histidine, and arginine—distinguishing it from other PTMs such as phosphorylation which have more limited targeting (Cohen and Chang 2018).

Beyond proteins, polynucleotides (i.e. DNA and RNA) are also targets of M/PARylation. First identified as a

CONTACT Michael S. Cohen cohenmic@ohsu.edu Department of Chemical Physiology and Biochemistry, Oregon Health and Science University, Portland, OR, USA

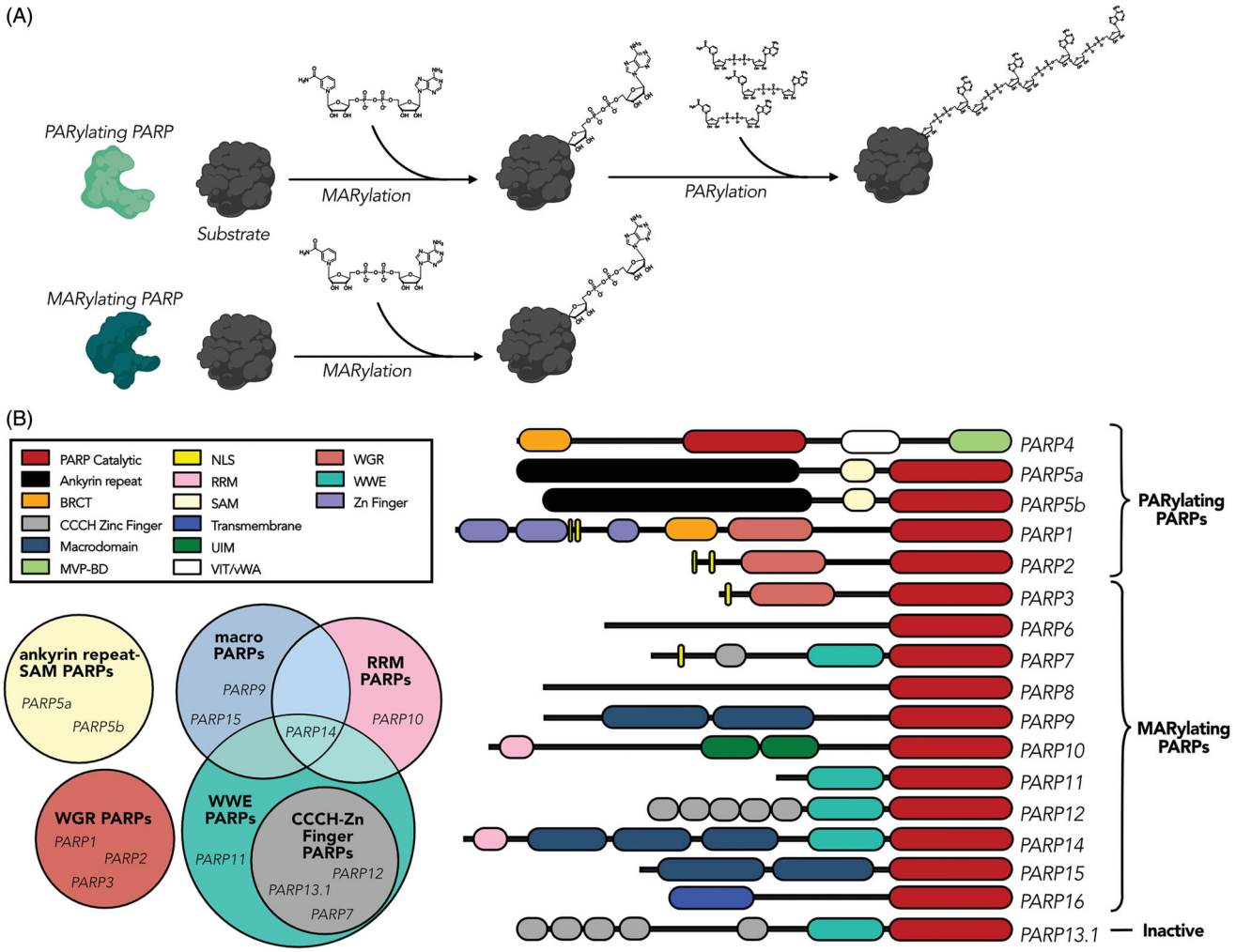

Figure 1. Summary of PARP activity and domain composition. (A) Schematic of PARP MARylation and PARylation reactions. (B) Graphical representation of the PARP domain architecture (not to scale).

target of MARylating toxins (cabbage butterfly toxin piersin-1 and bacterial toxin DarT) (Takamura-Enya et al. 2001; Jankevicius and Ahel 2016), DNA is also M/PARylated by mammalian PARPs (PARP1, 2, 3) (Munnur and Ahel 2017; Belousova et al. 2018; Zarkovic et al. 2018; Matta et al. 2020). While the toxins MARylate the nucelotide base—deoxyguanosine in the case of piersin-1 and deoxythymidine in the case of DarT—PARPs M/PARylate the terminal phosphate of DNA. Intriguingly, several PARPs (e.g. PARP10) MARylate selectively the terminal phosphate of RNA (Munnur et al. 2019). The function of DNA and RNA M/PARylation is unknown, but represents an exciting future direction in the PARP field.

PARPs play critical roles in essential cellular processes, including DNA repair, transcription, Wnt signaling, and innate immunity (Guo et al. 2019; Schwerk et al. 2019). Because of their roles in these essential cellular processes, PARPs are druggable targets. Indeed, several small molecules inhibitors (e.g., olaparib, talazoparib, rucaparib) of PARP1, which functions in DNA repair, are FDA-approved drugs for the treatment of cancer. While PARP1 has been the major focus of drug discovery efforts over the past several decades, emerging knowledge in the field demonstrates that other PARP family members are drug targets for cancer and other human diseases. Indeed, an inhibitor of PARP7, RBN-2397, is currently in Phase I clinical trials for the treatment of lung cancer (Clinal Trial # NCT04053673).

While the functions and downstream targets for most PARP family members, especially the MARylating PARPs, have not been elucidated, recent advances in mass spectrometry approach for accurately identifying the sites of M/PARylation, as well as the advent of selective small molecule inhibitors and antibody-based reagents for detecting M/PARylation, promise to accelerate our understanding of their physiological and pathophysiological functions. Reviews discussing these new advances have been

published recently (Luo and Kraus 2011, 2012; Bock et al. 2015; Gupte et al. 2017; Cohen and Chang 2018). In this review, we focus on our latest understanding of PARP regulation: from the control of PARP expression at the level of transcription and protein stability to subcellular localization and control of catalytic activity. We will discuss the role of the catalytic and regulatory domains of PARPs as well as auxiliary proteins in the aforementioned processes. Lastly, we will discuss the state-of-the-art regarding small molecule inhibitors that target regulatory domains of PARPs. Our goal is two-fold: (i) to provide an overview of themes and concepts in PARP regulation and (ii) to inspire further exploration in the burgeoning field of PARP biology.

Transcriptional regulation of PARP levels

Unlike PARP1, most PARP family members are expressed at low levels in cells under basal conditions (Nagaraj et al. 2011). The expression levels of several MARylating PARPs are controlled transcriptionally. This is most clearly exemplified by the transcriptional regulation of MARylating PARPs by type I and type II interferons (Table 1). The induction of these PARPs by interferons points to their role in immune signaling and host–defence against pathogens. This has proven to be the case for a number of MARylating PARPs: PARP12 is required for the degradation of viral proteins (Li et al. 2018); PARP11 regulates interferon response upon viral entry (Guo et al. 2019); and PARP10 suppresses viral proteolysis (Krieg et al. 2020).

Directly relevant to the current COVID-19 pandemic, coronavirus (CoV) infections induce the expression of several MARylating PARP family members. Treatment of cells with the coronavirus SARS-CoV-2 results in the upregulation of multiple MARylating PARPs, most notably PARP10, 12, and 14 (Blanco-Melo et al. 2020; Heer et al. 2020). Knockdown of PARP14 in a murine CoV model, mouse hepatitis virus (MHV), increases viral replication, suggesting PARP14 is antiviral (Grunewald et al. 2019). These results support the notion that MARylating PARPs regulate coronavirus infection and thus represent potential therapeutic targets for COVID-19.

Beyond interferons and viral infection, MARylating PARP expression can be induced by other stimuli. In some cases, different stimuli can induce the same MARylating PARP. For example, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), famously known as a contaminant of Agent Orange, induces the expression of PARP7 (a.k.a TiPARP) in hepatocytes (Ma et al. 2001). PARP7 knockout mice are more susceptible to liver toxicity induced by TCDD (Hutin et al. 2018). In non-small cell lung cancer cells (e.g. NCI-H647), epidermal growth factor (EGF) induces the expression of PARP7 (Mikule

Table 1. Interferon stimulation of PARP family member transcription.

Interferon stimulation of PARPs

PARP	IFN type	Transcription factors	Maximal fold change	Maximal fold change conditions
PARP1	I, II	NFKB, STAT3, STAT1	—	—
PARP2	I, II, III	IRF1, IRF8, STAT1	−3.0906599	In vitro, monocytes, type II interferon, 1.5 h, IFNgamma, 2000 IU/ml
PARP3	II	STAT1, STAT3, NFKB	4.248476	In vitro, blood-derived macrophages, Type II Interferon, 18 h, IFNgamma, 400 IU/ml
PARP4	I, II	IRF1, IRF7, IRF8, STAT1	8.209671	In vitro, Calu-3, type I interferon, 6 h, IFNalpha, 500 IU/ml
PARP5a	I, II	STAT1	−3.3468587	In vitro, blood-derived macrophages, type II interferon, 48 h, IFNgamma, 3 IU/ml
PARP5b	I	IRF1, IRF7, STAT1	3.2713284	In vitro, monocytes, type I interferon, 1.5 h, IFNalpha, 10,000 IU/ml
PARP6	—	—	—	—
PARP7	I, III	NFKB, STAT1, IRF1, IRF8	5.187552	In vitro, monocyte-derived macrophages, type I interferon 4 h, IFNbeta, 200 IU/ml
PARP8	I, II	STAT3, IRF8	4.58045	In vitro, human submandibular gland cell line, type II interferon, 48 h, IFNgamma, 1000 IU/ml
PARP9	I, II, III	ISRE, IRF, IRF1, IRF3, IRF7, IRF8, NFKB, STAT1, STAT3	23.324066	In vitro, cultured human umbilical vein endothelial cells, type II interferon, 24 h, IFNgamma, 200 IU/ml
PARP10	I, II, III	NFKB, NF1, STAT1, STAT3	12.379896	In vitro, fibroblast, type I interferon, 8 h, IFNalpha, 100,000 IU/ml
PARP11	I, II	STAT, STAT3, IRF1, STAT1	4.7312593	In vitro, monocytes, type I interferon, 1.5 h, IFNalpha, 10,000 IU/ml
PARP12	I, II, III	—	41.67324	In vitro, fibroblast, type I interferon, 5 h, IFNalpha, 1000 IU/ml
PARP13	I, II, III	IRF1, IRF7, IRF8, STAT1, STAT3, SIRT6	14.008896	In vitro, cultured human umbilical vein endothelial cells, type I interferon, 5 h, IFNbeta, 1000 IU/ml
PARP14	I, II, III	STAT1, STAT3, NF1, NFKB, IRF3, IRF7	35.133408	In vitro, cultured human umbilical vein endothelial cells, type II interferon, 24 Hours, IFNgamma, 200 IU/ml
PARP15	I	—	2.90491	In vitro, monocytes, type I interferon, 16 h, IFNbeta, 100 IU/ml
PARP16	II	NFKB, STAT3, IRF8	−2.272	In vitro, monocytes, type II interferon, 1.5 h, IFNgamma, 2000 IU/ml

Data from interferome V2.0 (Rusinova et al. 2013).

and Wang 2016). Knockdown of PARP7 in NCI-H647 decreases cell viability. While other PARP family members are not induced by either TCDD or EGF, it is likely that other stimuli induces these PARPs, the identification of which will provide insight into both PARP regulation and downstream function.

Regulation of PARP stability by catalytic activity

Beyond transcriptional regulation, accumulating evidence supports the notion that the protein levels in cells of certain PARPs are regulated by catalytic activity. This was first observed in cell-based experiments using an inhibitor (XAV939) of PARP5a/b (also known as Tankyrase1/2). Treatment of cells with XAV939 increases substantially PARP5a/b protein levels. Other, structurally unrelated PARP5a/b inhibitors also increase PARP5a/b protein levels (Ma et al. 2015).

How do these inhibitors regulate PARP5a/b protein levels in cells? One idea is that auto-PARylation of PARP5a/b regulates its turnover (Huang et al. 2009; Ma et al. 2015). It should be noted that the site of auto-PARylation has not been identified, precluding direct testing of this hypothesis. The turnover of auto-PARylated PARP5a/b (and other PARP5a/b targets) is controlled by ubiquitin degradation mediated by the ubiquitin E3 ligase RNF146. Binding of the Trp-Trp-Glu motif (WWE) domain of RNF146 to PAR conjugated to PARP5a/b stimulates the ubiquitin E3 ligase activity of RNF146, leading to ubiquitination of PARP5a/b and subsequent degradation. Inhibition of PARP5a/b with XAV939 (and related compounds) prevents auto-PARylation of PARP5a/b, which is expected to block RNF146-mediated ubiquitination of PARP5a/b and reduce the turnover of PARP5a/b.

Whether the protein levels of MARylating PARPs are regulated in the same manner is less clear, but recent studies point to a similar mechanism. Treatment of cells expressing GFP-tagged PARP11 with a selective small molecule inhibitor (ITK7) of PARP11 increases its protein levels (Kirby et al. 2018). Consistent with the inhibitor studies, a catalytically inactive mutant of PARP11 expresses at a much higher level than WT PARP11. This phenomenon is also observed for GFP-tagged PARP7 (Mikule and Wang 2016; Gomez et al. 2018). Since these studies involved GFP-tagged, overexpressed PARP fusion proteins, it will be important to evaluate the effects of small molecule inhibitors on endogenous protein levels of PARPs. These studies will be greatly facilitated by isoform-specific antibodies, of which few exist currently.

What is the mechanism of regulation of the protein levels of MARylating PARPs by catalytic activity? One possibility is that auto-MARylation of certain MARylating PARPs (e.g., PARP11) leads to recruitment of a ubiquitin E3 ligase that binds to and is allosterically activated by MAR, analogous to allosteric activation of RNF146 by PAR. While the well-studied MAR binding macrodomain (more on this below) has not been identified in ubiquitin E3 ligases, it is possible that unconventional MAR-binding domains exists. Indeed, several ubiquitin E3 ligases (e.g., ARIH2, MEX3B) contain RNA-binding domains that could moonlight as MAR binding domains. This mechanism may also apply to targets of MARylating PARPs. In support of this notion, the activity of PARP12 is required for ubiquitin-mediated degradation of the Zika virus proteins NS1 and NS3 (Li et al. 2018). Uncovering the mechanism by which MARylating PARP inhibitors regulate the protein levels of PARPs and their targets will provide insight into the function of MARylation.

Beyond the catalytic domain: regulatory domains of PARPs

The PARP family contains a panoply of regulatory domains that are critical for regulating PARP function in cells—from substrate targeting and subcellular localization to allosteric modulation and complex assembly. Given the heterogeneity of PARP domain architecture, it is helpful to organize PARP family members into groups based on their shared regulatory domains. We define six groups as follows: WGR PARPs, ankyrin repeat-SAM PARPs, macro PARPs, WWE PARPs, CCCH Zn finger PARPs, and RRM PARPs (Figure 1(b)). Some PARPs are in multiple groups whereas others fall outside of these groups as they contain domains that are either unique (PARP4 and 16) or uncharacterized domains (PARP6 and 8) (Figure 1(b)). Below we will briefly introduce the six PARP domain groups.

The most well-understood group is WGR PARPs: PARP1, 2, and 3. These PARPs share a WGR (Trp-Gly-Arg) domain, which is required for DNA-dependent activation of these PARPs (Langelier et al. 2014). PARP1 also contains additional domains: a BRCA1 C-terminal (BRCT) domain and three zinc (Zn) finger domains. The BRCT is commonly found in proteins that play crucial roles in the DNA damage response (DDR), like PARP1; and the Zn finger domains of PARP1 are required for binding to damaged DNA (Langelier et al. 2011).

The ankyrin repeat-sterile alpha motif (SAM) PARP group consists only of PARP5a and PARP5b. The ankyrin repeats are required for substrate targeting (Eisemann

et al. 2016); however, ankyrin binding alone is not sufficient to drive target PARylation as some proteins (e.g., GDP-mannose 4,6-dehydratase) that bind to the ankyrin repeats are not PARylated by PARP5a/b (Eisemann et al. 2019). The SAM domain mediates PARP5a/b polymerization, which is required for PARP5a/b PARylation activity (Mariotti et al. 2016; Riccio et al. 2016; Fan et al. 2018).

The macro PARP group—PARP9, 14, and 15—share a macrodomain (Amé et al. 2004; Perina et al. 2014). The macrodomain, originally identified in a histone variant, MacroH2A 1.1, binds MAR (Timinszky et al. 2009). Some macrodomain-containing proteins can remove MAR attached to either glutamate/aspartate in proteins or the 5′-phosphate of DNA/RNA; these include MacroD1, MacroD2, and Targ1 (Jankevicius et al. 2013; Rosenthal et al. 2013; Sharifi et al. 2013; Munnur et al. 2019). The macrodomains found in macro-PARPs are not catalytically active (Gregor et al. 2016), but are required for their function (more on this below).

The WWE PARPs—PARP7, 11, 12–14—make up the largest group. These PARPs and several ubiquitin E3 ligases (e.g., RNF146, discussed above) are the only protein families containing WWE domains. It is assumed that WWE PARPs bind PAR, but this has not been validated experimentally. This possibility, however, is fascinating because WWE-PARPs, except the pseudoPARP PARP13, catalyze MARylation and not PARylation, suggesting a potential cross talk between PARPs that catalyze MARylation and PARylation.

Three of the WWE PARPs—PARP7, 12, and 13—form another group called the CCCH Zn-finger PARPs group. CCCH Zn-fingers bind both hosts (Todorova et al. 2014; Schwerk et al. 2019) and viral RNA (Guo et al. 2004). Recent structural studies reveal how the CCCH Zn-finger of PARP13 binds and recognizes CG-rich RNAs (Meagher et al. 2019; Luo et al. 2020).

Finally, there are two PARPs that form the RNA recognition motif (RRM) PARP group: PARP10 and 14. Generally, multiple RRMs work in tandem to bind RNA with high affinity and sequence specificity. RRMs can also bind PAR; for example, the RRM domain of the RNA-binding protein NONO binds PAR generated by PARP1 during the DNA damage response (Krietsch et al. 2012). Given that the RRM domain of NONO also binds RNA, these results highlight context-dependent, RRM domain substrate competition. Whether RRMs of PARP10/14 bind PAR or RNA is not known. In addition to an RRM domain, PARP10 contains two ubiquitin interacting motifs (UIMs), which bind K63-linked polyubiquitin chains (Verheugd et al. 2013) and facilitate PARP10 interactions with PCNA (Nicolae et al. 2014).

Below, we will discuss in more detail how the regulatory domains of PARPs impact their catalytic activity, localization, and substrate targeting.

Subcellular localization of PARPs

PARP family members are subcellularly localized in mammalian cells. While the mechanisms regulating subcellular localization are generally poorly understood, it appears that both catalytic activity and regulatory domains are required for PARP localization under physiological and pathophysiological conditions.

In unstimulated cells, PARP7 localizes to the nucleus, as well as distinct subnuclear foci (MacPherson et al. 2013) (Figure 2). Mutation of the N-terminal CCCH Zn-fingers results in a loss of nuclear localization and an accumulation of PARP7 in the cytoplasm (Gomez et al. 2018). A catalytically inactive PARP7 mutant still localizes to the nucleus but no longer is found in subnuclear foci (Grimaldi et al. 2019). Similarly, mutations in the WWE domain of PARP7 disrupt subnuclear foci localization (Zhang et al. 2020). Together these results show that both the regulatory domains and catalytic activity are required for proper localization of PARP7 in cells.

Another MARylating PARP with distinct subcellular localization is PARP11. PARP11 localizes to the nuclear envelope (NE) in several cell types and MARylates nuclear pore complex proteins that reside there (Figure 2) (Meyer-Ficca et al. 2015; Carter-O'Connell et al. 2016). Intriguingly, NE localization of PARP11 is disrupted by inhibiting its catalytic activity with ITK7 (Kirby et al. 2018). Similarly, a catalytically inactive PARP11 mutant fails to localize to the NE. Much like PARP7, the WWE domain of PARP11 is also required for NE localization. Mutation of amino acids hypothesized to be required for PAR binding [based on studies with the WWE from RNF146 (Wang et al. 2012)] results in loss of NE localization (Meyer-Ficca et al. 2015). Taken together, these results suggest that, similar to PARP7, both the WWE domain and the catalytic activity of PARP11 are required for NE localization.

PARP16 is the only PARP family member that contains a tail-anchor (TA) domain. The TA domain of PARP16 is required for localization to the endoplasmic reticulum (ER) membrane (Di Paola et al. 2012; Jwa and Chang 2012). PARP16 is thought to be required for the unfolded protein response (UPR) (Jwa and Chang 2012). Interestingly, a PARP16 chimera, in which its TA domain is replaced with the TA of cytochrome b5, can still localize to the ER, but acts as a dominant negative by preventing activation of proteins in the ER stress pathway

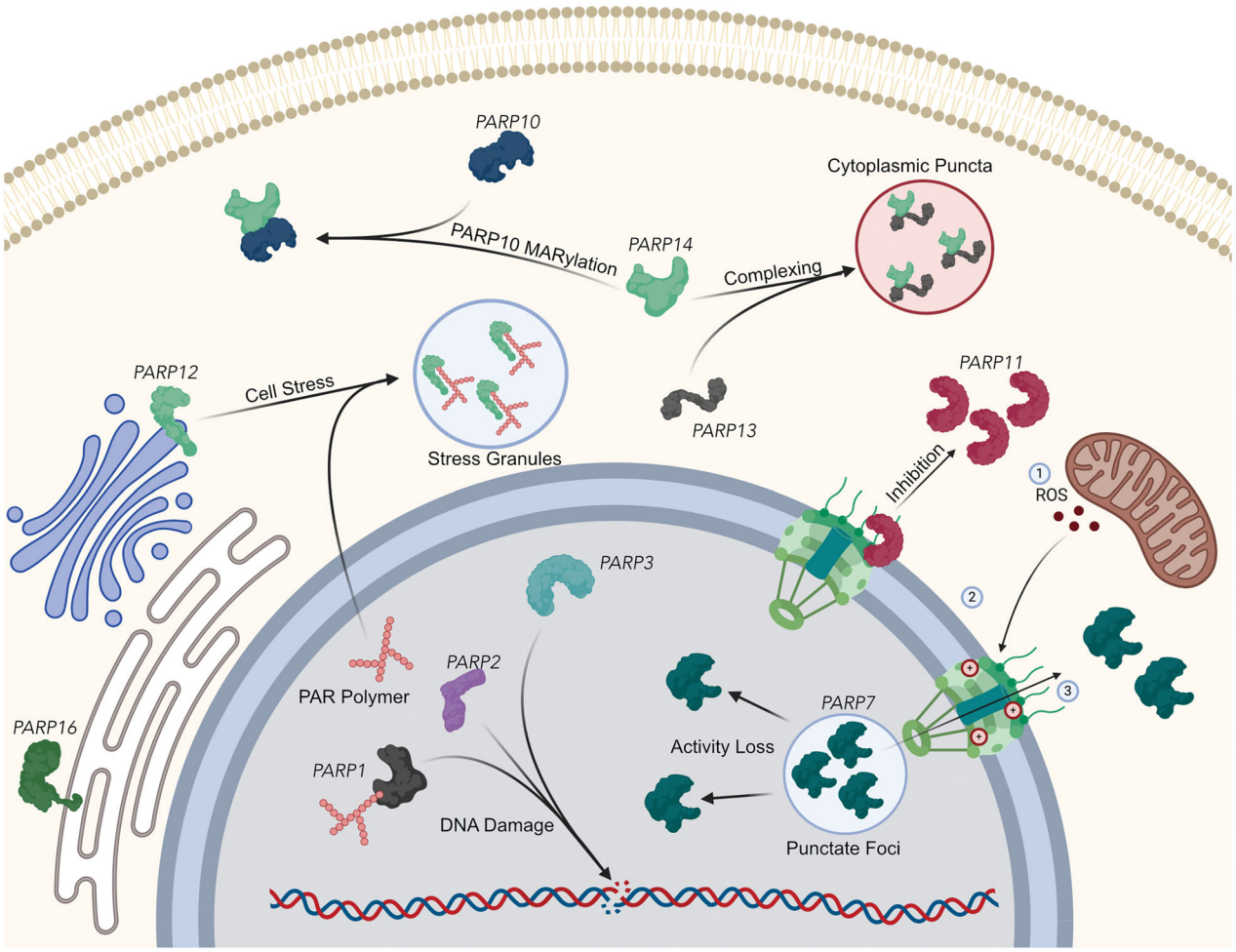

Figure 2. Subcellular localization of PARPs.

(Jwa and Chang 2012). These results suggest that the ER localization alone is not sufficient for PARP16 activation and that TA domain may directly impact PARP16 catalytic activity.

A theme emerging from the literature is that the localization of a PARP is context-dependent. For example, while the MARylating PARP PARP7 is primarily localized to the nucleus under basal conditions in mouse embryonic fibroblasts (MEFs), infection with Sindbis virus (SINV) causes PARP7 to accumulate in the cytoplasm (Kozaki et al. 2017). Treatment of cells with butylated hydroxyanisole, a reactive oxygen species (ROS) scavenger, partially blocks cytoplasmic accumulation of PARP7, suggesting ROS contributes to PARP7 nuclear-to-cytoplasmic transition. Additionally, treatment of cells with carbonyl cyanide 3-chlorophenylhydrazone (CCCP), an uncoupler of mitochondrial oxidative phosphorylation, induces cytoplasmic accumulation of PARP7 (Kozaki et al. 2017). Together, these results suggest that mitochondrial damage and/or dysfunction caused by SINV infection triggers PARP7 translocation.

Another example involves the MARylating PARPs PARP12 and PARP15. Under basal conditions, PARP12 localizes to the *trans*-Golgi network (TGN) (Catara et al. 2017) whereas PARP15 is localized to the nucleus (Leung et al. 2011). Upon induction of cell stress with sodium arsenite, PARP12 and PARP15 translocate to cytoplasmic stress granules (SGs) (Leung et al. 2011; Catara et al. 2017). In the case of PARP12, SG localization is dependent on the WWE and the CCCH Zn-finger domains of PARP12. The catalytic activity of PARP12 is not required for SG localization, but, intriguingly, depends on PAR generated by PARP1. This result highlights the crosstalk between MARylating and PARylating PARPs. PAR accumulates in SGs upon treatment with sodium arsenite and overexpression of PARG (cytoplasmic variant), an enzyme that breaks down PAR, inhibits SG assembly (Leung et al. 2011). One potential mechanism of PARP12 recruitment to SGs is via the binding of the WWE domain to PAR. While the mechanism regulating stress-dependent recruitment of PARP15 to SGs is not clear, it is possible that the macrodomain of PARP15, like the WWE domain of PARP12, binds PAR at

SGs. The idea of PAR acting as a dynamic recruitment scaffold and a biomolecular condensate is an exciting area of study in the PARP field. For a more thorough discussion on PAR scaffolding, we direct the reader to a recent review on this topic (Leung 2020).

Another emerging theme is the interaction between a catalytically active PARP family member and the catalytically inactive PARP family member (PARP13.1), in distinct subcellular compartments. PARP13.1 localizes to SGs upon oxidative stress (sodium arsenite treatment) (Leung et al. 2011) or viral infection (Law et al. 2019). It is thought that PARP13.1 interacts with other MARylating PARPs (e.g., PARP12/15) in SGs and may be a MARylation target. In the nucleus PARP13.1 can act as a scaffolding protein, regulating the interactions between the PARylating PARP1 and heat shock transcription factor (HSF1) during the DNA damage response (Fujimoto et al. 2017). PARP13.1 and PARP14 form a complex in cells and co-localize to cytoplasmic puncta. A chemical genetic approach for identifying direct targets of PARPs showed that PARP13.1 is MARylated by PARP14 (Carter-O'Connell et al. 2018). Whether or not PARP13.1 regulates the activity of PARP14 (or other catalytically active PARPs) in these subcellular compartments is not known. However, it is worth pointing out that the inactive, pseudokinase, STRAD, allosterically regulates the catalytic activity of the kinase LKB1 (Rajakulendran and Sicheri 2010). STRAD also stabilizes LKB1 by altering its subcellular localization (Veleva-Rotse et al. 2014). Perhaps PARP13.1 plays similar roles in the regulation of catalytically active PARPs.

Many of the studies described above are based on the overexpression of epitope-tagged variants and therefore must be interpreted with some caution. The dearth of commercially available, high quality isoform-specific antibodies for use in immunofluorescence studies has hampered efforts to evaluate the localization of endogenous PARPs. There is only one study that comprehensively evaluated the subcellular localization of endogenous PARPs using antibodies that, unfortunately are not commercially available (Vyas et al. 2013). In many cases, localizations using these antibodies are consistent with the overexpressed, tagged proteins. However, some discrepancies do exist—particularly, for PARP7 and PARP11. These antibodies were not validated using knockout cells, so it is unclear if they are recognizing their endogenous targets (which might be challenging considering their very low expression levels in cells). The only way to resolve this discrepancy is the development and validation (using knockdown or knockout approaches) of isoform-specific antibodies that can be widely distributed to the field.

Fine-tune control of the catalytic activity and substrate targeting of PARPs: the role of nucleic acids and auxiliary factors

Regulating the catalytic activity and substrate targeting of an enzyme is critical for cellular homeostasis. Indeed, hyperactivation or mistargeting of an enzyme can have deleterious consequences. Among the PARP family members, PARP1 is most well understood regarding the regulation of catalytic activity and substrate targeting. Under basal conditions, PARP1 exhibits low catalytic activity. PARP1 activity increases several thousand-fold upon binding to damaged DNA (Langelier et al. 2018). The low catalytic activity under basal conditions is because the NAD^+ binding pocket is occluded by an alpha helix (αF) in the helical domain (HD) (Langelier et al. 2018). Upon DNA damage, the Zn-finger domains and the WGR domain of PARP1 bind to damaged (e.g., single-strand breaks) DNA, inducing an allosteric change that relieves the αF-mediated block of NAD^+ binding. Hydrogen deuterium exchange experiments support the notion that this relief in blocking NAD^+ binding is due to a partial unfolding of αF (Dawicki-McKenna et al. 2015). Deletion of the HD, or mutations in the WGD–HD interface, in PARP1 uncouples this allosteric regulation, resulting in a constitutively active enzyme (Dawicki-McKenna et al. 2015). Together these results demonstrate how the regulatory domains impact PARP1 catalytic activity via allosteric regulation of DNA binding.

This type of allosteric regulation is relevant to other DDR-PARPs. Mutations in the WGR-HD interfaces of PARP2 and PARP3 result in a loss of DNA-dependent activation (Langelier et al. 2014). While the overall mechanism of DNA-dependent allosteric regulation of DDR-PARPs is similar, there are some differences. While PARP1 can be activated by several types of DNA damaged structures, PARP2 and PARP3 are activated specifically by 5′-phosphorylated DNA breaks. Additionally, PARP2 and PARP3 do not contain Zn-finger domains so allosteric regulation for these DDR-PARPs is dependent only on the WGR and HD domains (Langelier et al. 2014).

In addition to damaged DNA, small nucleolar (sno) RNAs (e.g., *SNORA37*) are activators of PARP1. The binding of *SNORA37* promotes PARP1-mediated PARylation of the RNA helicase DDX21 (Kim et al. 2019). Intriguingly, not all snoRNAs (e.g., *SNORA15*) bind and activate PARP1, supporting the notion that the

interaction between snoRNAs and PARP1 is highly specific. A recent study challenged the finding that the catalytic activity of PARP1 is stimulated by snoRNAs (Nakamoto et al. 2019). However, a subsequent study showed that *in vitro* transcribed *SNORA37*, but not *SNORA15*, activates highly purified PARP1 *in vitro* (Huang et al. 2020). snoRNA and DNA activate PARP1 to a similar extent. Critically, treatment with RNAse abolishes selectively snoRNA-activated PARP1 whereas treatment with DNAse has no effect. In contrast, DNAse treatment abolishes DNA-dependent activation of PARP1. Together these results provide strong support that in addition to DNA, certain snoRNAs stimulate PARP1 catalytic activity.

PAR—when attached to PARP1—can act as a recruitment scaffold for PARP1 targets. In one example, the C-terminal domain (CTD) of the tumor suppressor p53 binds PAR, which is required for p53 PARylation by PARP1 (Fischbach et al. 2018). Attaching the CTD of p53 to a protein that is not normally PARylated results in PARP1-mediated PARylation, demonstrating that the CTD is sufficient to dictate target PARylation. The CTD of p53 is a basic, intrinsically disordered domain, which is found in many human proteins. It will be interesting to determine if intrinsically disordered domains of other proteins also govern target PARylation by PARP1. In another example, the C-terminal RNA binding domain of DDX21 binds PAR, which leads to its PARylation by PARP1 (Kim et al. 2019). Using chemical genetic approaches for identifying direct targets of PARPs, we and others found that many DDX proteins are targets of PARP1 and PARP2 (Carter-O'Connell et al. 2014; Gibson et al. 2016). It will be important in future studies to determine if PARylation of these DDX targets are similarly regulated by PAR binding to their RNA binding domains.

Exciting new findings demonstrate that histone PARylation factor 1 (HPF1)—which we refer to here as an auxiliary factor—can influence PARP1 substrate and amino acid targeting (Gibbs-Seymour et al. 2016; Bonfiglio et al. 2017). Early work showed that HPF1 limits PARP1 hyper-automodification and promotes trans-modification of histones (e.g. H3) during the DNA damage response (Gibbs-Seymour et al. 2016). Mass spectrometry analysis revealed, surprisingly, that HPF1 directs serine M/PARylation of histones as well as PARP1 itself (Figure 3(a)). Proteome-wide mass spectrometry analysis further supports that all PARP1-mediated serine M/PARylation depends on HPF1 and occurs on serines often preceded by a lysine (referred to as the "KS motif") (Bonfiglio et al. 2017). In the absence of HPF1, PARP1 auto- and trans-PARylates on glutamate/aspartate amino acids (Gibson et al. 2016; Figure 3(a)). Critically, serine and glutamate/aspartate M/PARylation sites are differentially regulated by distinct M/PAR hydrolases: ARH3 removes selectively M/PAR attached to serines (Fontana et al. 2017), whereas the macrodomains MacroD1/2 and TARG1 (Slade et al. 2011; Jankevicius et al. 2013; Rosenthal et al. 2013; Sharifi et al. 2013) removes selectively M/PAR attached to glutamate/aspartate. This has important implications for the dynamics of serine versus glutamate/aspartate M/PARylation in cells.

HPF1 is also an auxiliary factor for PARP2 and promotes serine targeting in PARP2 substrates. Mutagenesis and inhibitor studies support the notion that HPF1 binding to PARP1/2 is dependent upon the local unfolding of αF, which occurs upon allosteric activation via DNA binding to the regulatory domains of PARP1/2 as discussed above (Suskiewicz et al. 2020). The crystal structure of the catalytic domain of PARP2 bound to HPF1 was solved recently, providing insight into how HPF1 acts as an auxiliary factor to impact PARP1/2 substrate and serine amino acid targeting (Suskiewicz et al. 2020). The co-crystal structure shows that HPF1 forms a joint active site with PARP2 (Figure 4(a)). Of particular interest is glutamate 284 (Glu284), which based on a modeled NAD^+, is near the nicotinamide ribosyl C1 position. Glu284 could potentially act as a general base to deprotonate the incoming serine nucleophile which will attack the ribosyl C1 position in an SN_2-like mechanism. Mutation of Glu284 to an alanine abolishes serine-M/PARylation of histone H3, but does not affect auto-PARylation of PARP1 (presumably on Glu/Asp).

In addition to the interactions described above, HPF1 forms extensive interactions with a region of PARP2 known as the activator (A)-loop (Figure 4(a)). Based on comparisons to the well-studied bacterial ADP-ribosyltransferases, the A-loop of PARPs is thought to contribute to substrate and amino acid targeting (Cohen and Chang 2018). The A-loop is one of the most structurally diverse elements in terms of amino acid composition and length across the PARP family (Figure 4(b–d)). While consensus sequence analysis of the A-loop of MARylating PARPs shows that some positions are generally conserved—for example the "floor" position (amino acid that sites at the base of the nicotinamide subsite in the NAD^+ binding pocket) is a hydrophobic amino acid and the −2 position relative to the floor is a proline—most positions in the A-loop are not (Figure 4(d)). This diversity could impact which substrates are targeted. It will be interesting to determine if, similar to PARP1/2, other PARPs, especially the

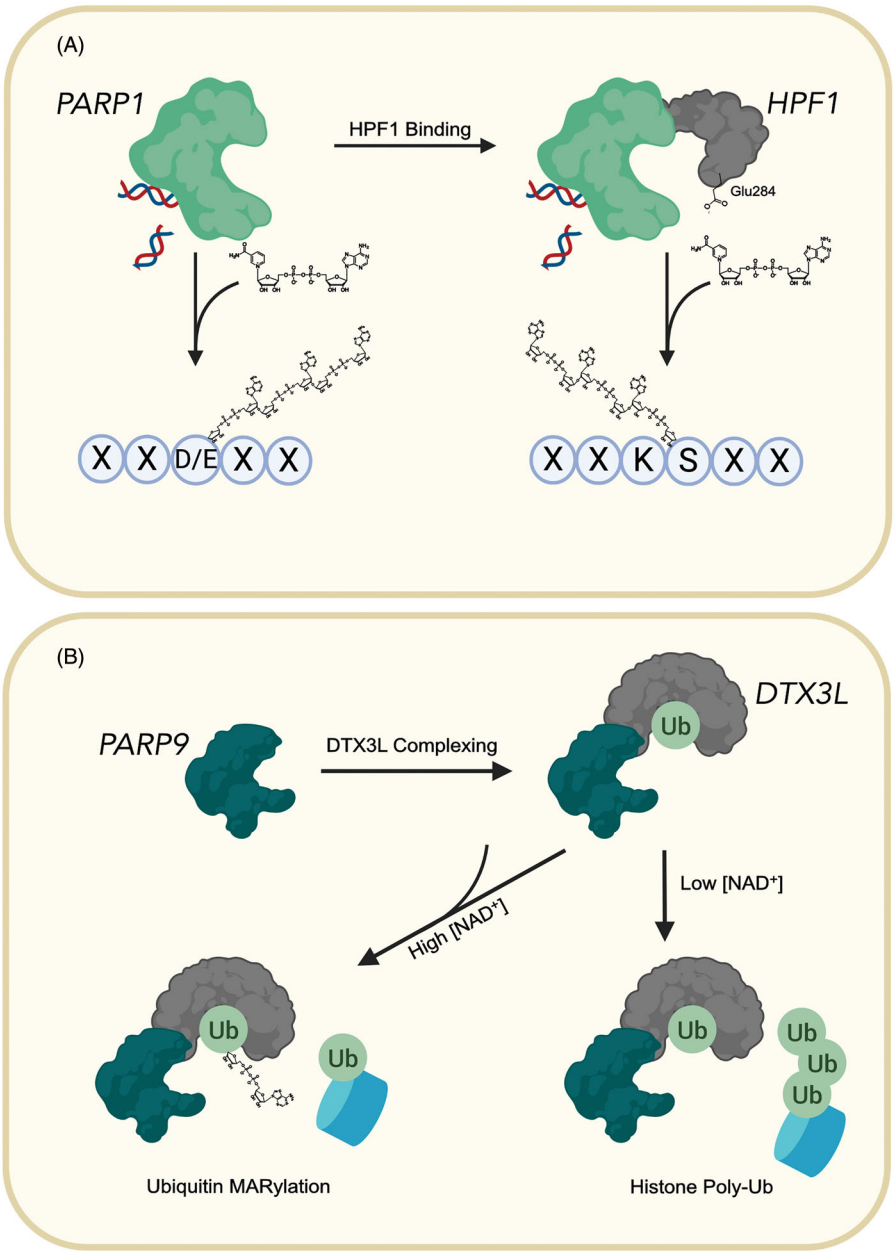

Figure 3. Control of PARP activity by auxiliary factors. (A) Binding of HPF1 to PARP1 regulates substrate recognition and amino acid target specificity. (B) PARP9 is activated by binding to DTX3L and further controlled by NAD^+ concentrations.

MARylating PARPs, have auxiliary factors that bind to the A-loop and influence substrate and amino acid targeting. Taken together, these studies show that HPF1 is an auxiliary factor that can regulate PARP1/2 substrate and amino acid targeting, in part, by forming a joint active site with PARP1/2. It will be interesting to determine if other auxiliary factors of PARP1/2 exist and whether these direct PARP1/2 toward M/PARylation of other amino acids.

Beyond PARP1/2, the MARylation activity of PARP9 is regulated by auxiliary factor binding. PARP9 does not auto-MARylate like other MARylating PARP family members and was assumed to be inactive. However, binding of the E3 ligase DTX3L to PARP9 stimulates its MARylation activity toward ubiquitin (Yang et al. 2017) (Figure 3(b)). The PARP9/DTX3L complex MARylates the carboxyl group of the C-terminal glycine of ubiquitin. Because the carboxyl group is required for conjugation of ubiquitin to protein substrates, MARylation of the group is expected to block ubiquitin conjugation. Indeed NAD^+ inhibits the E3 ligase activity of PARP9/DTX3L on histone substrates. It should be noted that complete inhibition of histone ubiquitination by PARP9/DTX3L occurs at ~1 mM NAD^+, which is at least

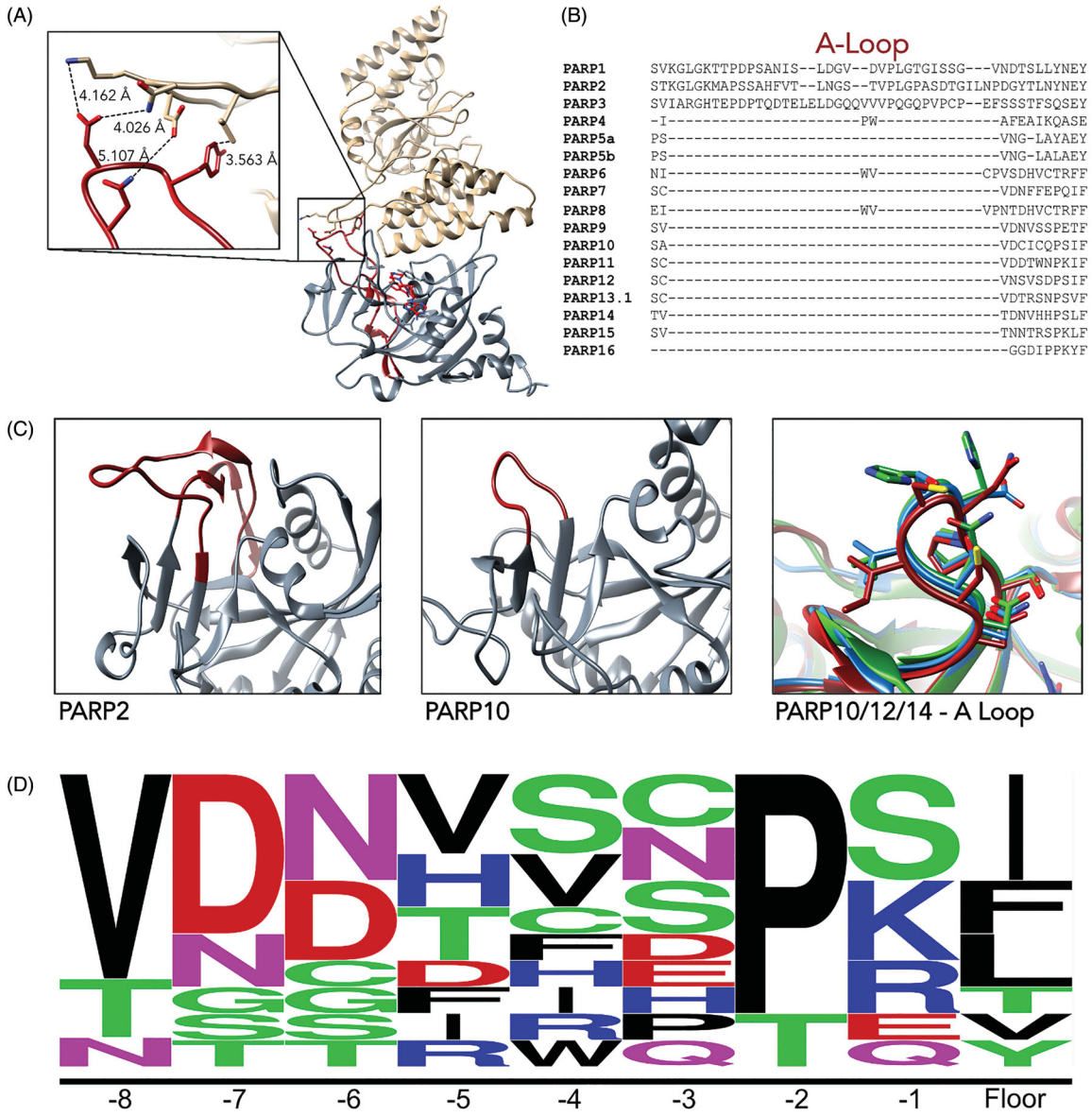

Figure 4. PARPs contain A-Loop domains that vary in structure and sequence. (A) PARP2 (gray) interacts with HPF1 (tan) via the A-Loop domain (red). (PDB: 6TX3). (B) PARP A-Loops vary across the family in length and amino acid make-up. Alignment created in Clustal Omega (Abio Madeira et al. 2019). (C) Structural comparison of A-Loop variations in structure and amino acid sequence. Left: PARP2 (gray) with A-Loop (red). Center: PARP10 (gray) with A-Loop (red) Right: A-Loops of PARP10 (PDB: 5LX6) (red) PARP12 (PDB: 2PQF) (blue) PARP14 (PDB:5NQE) (green). (D) Frequency plot of MARylating PARP A-Loops generated by WebLogo (Crooks et al. 2020) (see colour version of this figure at www.tandfonline.com/ibmg).

10-fold higher than the concentration of NAD^+ in cells (Cambronne et al. 2016). Notably, there is no known hydrolase for C-terminal carboxyl MARylation and the ultimate fate of MARylated ubiquitin is unclear. Nevertheless, these results show that the activity of PARP9 can be augmented by auxiliary factor binding.

These studies highlight the importance of identifying binding proteins of PARPs that may act as auxiliary factors to regulate substrate and amino acid targeting. Recently, a proximity labeling approach (referred to as BioID) was used to identify intracellular interactors of PARP14 (Carter-O'Connell et al. 2018). Whether any of these binding partners regulate PARP14 remains to be determined. Family-wide application of BioID or similar approaches under basal and activated (e.g., cell stress, viral infection) states will provide insight into the regulation of PARP activity by auxiliary factors.

Final thoughts and closing remarks

PARP regulatory domains and auxiliary factors are critical for the control of PARP activity and response to cellular stimuli. Current FDA approved therapeutic strategies toward PARPs only target the NAD^+ binding

site in the catalytic domain. We point readers to the following review for the development of catalytic PARP inhibitors (Kirby and Cohen 2019). However, the targeting of the PARP regulatory domains or auxiliary factors may prove to be a tunable therapeutic approach and will undoubtedly generate chemical tools that will illuminate PARP biology in ways that are distinct from active site-targeted inhibitors. Indeed, some of these small molecule modulators are already in the works. An inhibitor of the macrodomains of PARP14 was recently described, and this inhibitor prevents the recruitment of PARP14 to sites of DNA damage (Schuller et al. 2017). In another example, a fragment-based approach identified several small molecule lead compounds as antagonist of the ankyrin repeats of PARP5a (Pollock et al. 2019). Lastly, small molecules that bind the minor groove of DNA are effective at disrupting PARP1's interaction with DNA and thus preventing PARP1 activation (Kirsanov et al. 2014). These studies demonstrate the potential of nonactive site targeted small molecule modulators of PARPs.

PARP regulatory domains may serve as useful starting points for targeting PARP domains that are shared across proteins, such as the macrodomain, which are required for viral activity in coronaviruses (McPherson et al. 2017; Grunewald et al. 2019) or other pathogenetic effectors that "hijack" the human ADP-ribosylation system. Additionally, PARP regulatory domains can activate PARPs (Langelier et al. 2018; Huang et al. 2020). This opens the door to targeting these domains with small molecule agonists to activate PARP family members.

We hope this review provides a snapshot into the regulation of PARPs in cells. While we have learned quite a bit about the function of PARP regulatory domains and identified a few auxiliary factors that can modulate PARP activity, many questions remain: (1) are there other auxiliary factors that activate, increase or inhibit PARP activity? (2) to what extent does PARP crosstalk exist in cells? and (3) what are the functions of the understudied PARP regulatory domains, such as the RRM domains of PARP10 and PARP14? Answers to these questions will provide a more detailed understanding of PARP biology and generate, potentially, new avenues of therapeutic inquiry. New tools are required to answer these questions; these include: Isoform- and MARylation-specific antibodies, reagents for monitoring the activities of individual PARPs in intact cells, selective small molecule inhibitors for every PARP family member, and small molecules that bind to the regulatory domains of PARPs. We look forward in excited anticipation for the development of these and other tools that promise to propel the field forward.

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Disclosure statement

The authors report no conflict of interest.

ORCID

Daniel J. Sanderson http://orcid.org/0000-0002-0516-814X
Michael S. Cohen http://orcid.org/0000-0002-7636-4156

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