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PAR recognition by multiple reader domains of PARP1 allosterically regulates the DNA-dependent activities and independently stimulates the catalytic activity of PARP1

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ABSTRACT

Poly(ADP-ribosyl)ation is a post translational modification, predominantly catalyzed by Poly(ADP-ribose) polymerase 1 (PARP1) in response to DNA damage, mediating the DNA repair process to maintain genomic integrity. Single strand (SSB) and double strand (DSB) DNA breaks are bonafide stimulators of PARP1 activity. We identified that, in addition, single strand (ss) DNA also binds and stimulates the PARP1 activity. Poly(ADP-ribose) (PAR) is chemically similar to ssDNA. However, PAR mediated PARP1 regulation remains unexplored. Here, we report ZnF3, BRCT and WGR, hitherto uncharacterized, as PAR-specific reader domains of PARP1. Surprisingly, these domains recognize PARylated protein with a higher affinity compared to PAR, but do not bind to DNA. Conversely, N-terminal domains, ZnF1 and ZnF2, of PARP1 recognize DNA but not PAR. Further competition binding studies suggest that PAR binding, allosterically releases DNA from PARP1. Unexpectedly, PAR showed catalytic stimulation of PARP1 but hampers the DNA dependent stimulation. Altogether, our work discovers dedicated PAR and DNA reader domains of the PARP1, and uncovers a novel mechanism of allosteric stimulation of the catalytic activity of PARP1 but retardation of DNA-dependent activities of PARP1 by its catalytic product PAR.

Keywords: Poly(ADP-ribose) polymerase, DNA repair, PAR reader domains, Poly(ADP-ribose), Allosteric regulation

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INTRODUCTION

Poly(ADP-ribose) polymerase 1 (PARP1), an abundant nuclear enzyme often referred to as "genome guardian", plays a crucial role in maintaining genome integrity (1). It is involved in several cellular functions, including DNA damage repair (2–6), regulation of chromatin structure (7–9), transcription (10–12) and apoptosis (13, 14). PARP1, the founding member and the most studied enzyme among the PARP family, is a multi-domain protein consisting of three zinc finger domains, ZnF1, ZnF2 and ZnF3 at the N-terminal, followed by the BRCT (Breast cancer associated C-terminal) domain and the WGR (Trp-Gly-Arg rich) domain in the central region, and the catalytic (CAT) domain at the C-terminal, which comprises the helical sub-domain (HD) and the signature ADP-ribosyl transferase (ART) sub-domain (Figure 1A). PARP1 has been shown to recognize different types of damaged DNA by different combinatorial sets of ZnFs. The ZnF1 and ZnF3 together bind to a double strand break (DSB) whereas ZnF1 and ZnF2 bind to a single strand break (SSB) DNA (15–19). However, the involvement of PARP1 domains, either individual or in combination, has not been studied extensively.

Further, recognition of damaged DNA by zinc finger domains of PARP1 induces a global conformational change in the protein, i.e., spatial re-organization of the domains, which facilitates the positioning of CAT domain close to the automodification (AD) region, which comprises the C-terminal region of BRCT domain and the loop connecting the BRCT and WGR domains (20, 21). PARP1, then, catalyzes a series of covalent addition of ADP-ribose (ADPr) on the AD region using NAD^+ as a co-factor to form a long, linear or branched, chain of poly(ADP-ribose) also referred to as PAR, consisting of >200 units of negatively charged ADPr, and the phenomenon being known as PARylation (Figure 1B) (16, 22). PARP1 catalyzes the PARylation activity not only on itself (self-PARylation) but also on the other target proteins (trans-PARylation) on the target residues including aspartate, glutamate, lysine, serine, arginine, etc. (23–27).

PAR serves as a docking platform for recruitment of DNA damage repair proteins. Several domains in various proteins have been identified to bind PAR. Besides the classical, well characterized PAR reader modules, macro domain, WWE, PBZ (PAR binding Zinc finger), PBM (PAR binding motif), from PAR hydrolysing enzymes, there are other domains which have been reported to recognize PAR. These include FHA (Fork head associated) domain, OB (oligonucleotide or oligosaccharide binding fold), BRCT which recognize ADPr, etc. (28, 29). Recently, PARP2 was reported to recognize PAR through unstructured N-terminal region, which also regulates its activity (30).

Both, PARP1 and PARP2, are allosterically regulated by DNA binding, resulting in activation of the enzymatic activity of the proteins (31, 32). Almost all kinds of DNA, such as, DSB, SSB, overhangs, cruciform and hairpin, with or without 5'-Phosphate (5'P), are able to stimulate the activity of PARP1, although to different extents (33). PARP1 is also known to be activated upon binding to different types of RNA like snoRNA, pre-mRNA and therefore involved in RNA biogenesis (34), alternative splicing of mRNA (35), etc. Although the degree of activation of PARP1 upon RNA binding is less compared to DNA binding (30). Also, one of the study shows that there are RNAs which could bind non-specifically to PARP1 and PARP2 but could not activate them (36). Proteins such as histones (37), HPF1 (38), mH2A1.1 (39), HMGN1 (40), XPA (41), NEIL1 (42), OGG1 (43), DDB2 (44), p53 (45), ERK2 (46), Sam68 (47), YB-1 (48), C12orf48 (49), etc. physically interact with the PARP1 and regulate the

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PARP1 activities. In addition, interaction of PARP1 with other proteins or another PARP1 molecule is strengthened significantly in presence of DNA (42, 43) or PAR (50) owing to the involvement of additional protein domains. Since chemical nature of PAR polymer is very similar to nucleic acid molecules, like RNA / DNA (Supplementary Figure 1), we hypothesize that domains which recognize DNA may also bind to PAR.

Here, using biophysical approaches, we have established the role of different domains of PARP1, individual and in combination, in recognition of PAR and DNA. Our studies show that different sets of PARP1 domains mutually and selectively recognize the PAR, and damaged and single strand DNAs. Our work also reveals that PAR partially inhibits the DNA dependent catalytic activity of full-length PARP1 ($PARP1_{FL}$) and also acts as a stimulator of $PARP1_{FL}$ activity. Further, we elucidate the possible mechanism of DNA dislodging from PARP1 upon PAR binding. Our studies also establish the single stranded DNA (ssDNA) as an additional regulator of PARP1 which may have further implications yet to be explored.

MATERIALS AND METHODS

Cloning, expression and purification of $PARP1_{FL}$, variants of PARP1, PARP2 and PNC1

The full length human PARP1 (aa 1-1014) cDNA in pET28-a(+) has been purchased from GenScript, USA.. $PARP1_{FL}$ (aa: 1-1014), ZnF1-BRCT (aa: 1-494), WGR-CAT (aa: 518-1014) and individual domain constructs were generated by cloning the sequence in bacterial expression vector pRSFDuet-1 vector between SacI and XhoI restriction sites. Domain length of individual constructs is same as given in Langelier et al., 2011 (51). *E. coli* Rosetta 2 (DE3) were transformed with the constructs for expression. An hour before inducing the culture with 0.2 mM IPTG, 100 μM $ZnSO_4$ was added. Culture was induced at 16˚C for 16-18 h, then harvested. The pellet was resuspended in lysis buffer containing 500mM NaCl, 50mM HEPES pH 7.5, 20mM Imidazole and 3mM β-mercaptoethanol Protease inhibitor cocktail tablets (Pierce™ Protease Inhibitor, Thermo Scientific) were added in case of $PARP1_{FL}$ only. The cell suspension was sonicated, the lysate was clarified by centrifugation and the proteins were purified in 3-steps. First, supernatant was passed through Ni-NTA affinity column (5ml, HisTrap column, GE Healthcare), followed by ion exchange Heparin column (HiTrap Heparin HP, GE Healthcare) with salt gradient of (250-1000mM NaCl for $PARP1_{FL}$ and 50mM-1000mM NaCl for all other constructs) and finally through gel filtration (16/600 200pg, GE Healthcare) column pre-equilibrated with buffer (150mM NaCl (for $PARP1_{FL}$) and 50mM NaCl (for all other constructs), 10mM HEPES, 2% Glycerol and 2mM β-mercaptoethanol). Purified proteins were concentrated, flash frozen and stored at -80˚C.

The full length human PARP2 isoform 2 cloned in pET28-a(+) was generously gifted by Dr. John Pascal. PARP2 was expressed in *E. coli* BL21(DE3) at conditions same as $PARP1_{FL}$ and purified using Ni-NTA affinity and ion-exchange chromatography using the protocol mentioned for $PARP1_{FL}$.

PNC1 from Saccharomyces cerevisiae cloned in pET41-b(+) was obtained as a generous gift from Prof. Antonio Barrientos. *E. coli* Rosetta 2(DE3) were transformed with the construct for expressing the protein. Culture was induced with 0.1 mM IPTG at 18°C. Cells were harvested after 16 h and resuspended in buffer containing 50mM Tris-HCl pH 8.0, 500mM NaCl, 10mM imidazole and 10% glycerol. Cell suspension was sonicated, and lysate was centrifuged at 4°C. Obtained supernatant was loaded onto Ni-NTA affinity column (5ml, HisTrap column, GE Healthcare) and eluted by step

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gradient of 10-400mM Imidazole in the same buffer. Fractions were pooled and passed through gel filtration column (16/600 75pg, GE Healthcare) pre-equilibrated with 20mM Tris pH 8.0, 150mM NaCl, 1.5 mM β-mercaptoethanol and 1% glycerol. Purified PNC1 was concentrated, flash frozen and stored at -80˚C. (52).

PAR generation and purification and fractionation

PAR was generated by performing automodification reaction of $PARP1_{FL}$, cleaved from the protein and purified according to the protocol given in Lin et al., 2018 (53). Obtained PAR was diluted with 20mM Tris-HCl buffer pH 7.5 and was loaded into Resource Q anion exchange 1ml column (GE Healthcare) for fractionation with a linear gradient of 0 - 1M NaCl at 0.25ml/min flow rate, with FPLC UV filter set at 254nm. Obtained separate fractions were precipitated by adding 1/10th volume of 3M sodium acetate pH 5.2, and 2.5 times volume of ice-cold 100% ethanol. After incubating overnight in -20˚C, the precipitates were separated by centrifugation at 15000g for 30 min at 4˚C. The pellet was washed with 1ml of 70% ethanol twice by centrifugation at 15000g for 10min. Pellet was air dried and resuspended in 20µl of autoclaved distilled water. Concentration was calculated by taking the absorbance at 258nm and taking the molar extinction coefficient of ADPr 13.5mM^{-1} cm^{-1}.

Nucleic acids generation and annealing

HPLC purified lyophilized DNA oligonucleotides (listed in Supplementary Table 1) and complementary strands were purchased from oligo synthesis facility of W.M. Keck Foundation, Yale School of Medicine, USA and dissolved in a buffer containing 10 mM Tris-Cl (pH 7.5), 50 mM NaCl and 3 mM $MgCl_2$. DSB DNA as prepared by mixing the complementary strands in an equimolar ratio. A dumbbell-forming 45-mer DNA oligonucleotide was used to make SSB DNA. DSB and SSB DNA were kept for annealing at 95°C for 5 minutes and cooled at 4°C for overnight. ssDNA and 5'P-ssDNA were dissolved in same buffer and used as it is.

Generation of PARylated PARP2

PARylated PARP2 was generated using the same automodification protocol as used for $PARP1_{FL}$ with incubation time of 30min, after which the reaction mixture was passed through the salt exchange column (HiPrep 16/10 Desalting, GE Healthcare) with FPLC UV filter set at 254nm and then concentrated using 10kDa concentrator. Concentration was calculated by subtracting the measured absorbance at 258nm and 280nm of the final concentrated sample and before keeping for automodification reaction and taking the molar extinction coefficient of ADPr 13.5mM^{-1} cm^{-1}.

Isothermal Titration Calorimetry (ITC) binding studies

ITC binding studies were carried out at 25˚C using LV Affinity ITC (TA Instruments). Reference cell was filled with deionized water. For all binding studies, DNA (5-10 µM) (Supplementary Table 1), PAR (10-20 µM) or PARylated PARP2 (10-20 µM, concentration according to 10 mer PAR) were added in cell (350µl) and PARP1 domains (0.1-0.3 mM) were taken in syringe. 20 consecutive injections of 2.5 µl at an interval of 120s were performed while stirring the cell content at 125rpm. Analysis and processing of the binding isotherm was performed using NanoAnalyse software provided by TA instruments. For binding study with ADPr, 0.1mM of either BRCT, WGR or ZnF1-BRCT was taken in cell and 1mM ADPr in syringe.

Fluorescence Polarization (FP) studies

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Fluorescence polarization DNA binding studies were performed in gel filtration buffer using SpectraMaxID5 (Molecular devices). Fluorophore, 5-FAM, labelled DNAs (Supplementary Table 1) were used for binding and competition binding studies. For DNA binding studies, 20nM fluorophore labelled DNA was used and protein concentration was varied from 0.2 nM to 2 µM). For the competition binding assay, 40 nM of protein was incubated with the 20nM of probe DNA and PAR was added in varying concentration of 2.5 nM to 5 µM at room temperature for 45 min. The FP values were plotted against the variable entity into a nonlinear regression model. The *Ki* were calculated from Binding-Competitive model in GraphPad Prism. The reported *Ki* and K_D represent an average of three independent experiments.

PNC1-OPT assay

PNC1-OPT assay was performed to measure the amount of NAD+ consumed during PARylation by $PARP1_{FL}$ (54). Reactions were performed in a 96-well plate with one reaction in the absence of NAD+ to account for background fluorescence. PARylation reaction was performed at room temperature by adding 1µM $PARP1_{FL}$, DNA 1µM and/ or PAR -10 µM) and 5mM nicotinamide in buffer (25 mM HEPES pH 8.0, 140 mM KCl, 8 mM MgCl2, 50 µg/ml BSA, 0.02 mM TCEP, 4% glycerol, and 11.4 mM ME). The reactions were quenched with 5 mM benzamide after 30s, 45s, 1min, 2min, 5min, 10min, 20min and 30min. Purified yPnc1 enzyme was added at 20 µg/ml to the quenched reactions. The reactions were incubated at 37°C for an hour. During the incubation period, OPT developer reagent (10 mM ortho-pthalaldehyde (OPT), 30% ethanol and 10 mM dithiothreitol) was prewarmed at 37◦C in the dark. Following the incubation period, 50 µl of OPT developer reagent was added to each reaction under dim light. The reactions were incubated at room temperature for 1 hour at 45 rpm on an orbital shaker. The plates were transferred to a plate reader (EnSpire™ Multimode Plate Reader by PerkinElmer, Inc.) with monochromators set to excitation at 420 nm and emission at 450 nm. Using the arbitrary fluorescence unit (AFU) readings for each reaction, the net fluorescence was calculated for each sample by subtracting the fluorescence of the control reaction (−NAD+) from the experimental reaction, F(corrected) = F(sample) – F(−NAD+). The resulting value is proportional to the amount of nicotinamide produced during the PARylation reaction and therefore the amount of NAD+ consumed. Serially diluted nicotinamide was used as a standard curve. Data analysis was performed from three independent reactions. Error bars indicate standard deviation of three independent experiments.

RESULTS

Binding studies of Zn-finger domains of PARP1 to PAR

Our isothermal titration calorimetry (ITC) binding studies of PAR with individual zinc finger domains of PARP1 showed that among the zinc fingers (ZnF), ZnF3 exhibited a highest binding affinity towards PAR with a molar dissociation constant (K_D) of 2.8 µM. Compared to ZnF3, ZnF1 showed ~53 times less binding affinity (K_D ~150 µM), and ZnF2 exhibited even weaker binding (K_D could not be determined) (Figure 1C-E).

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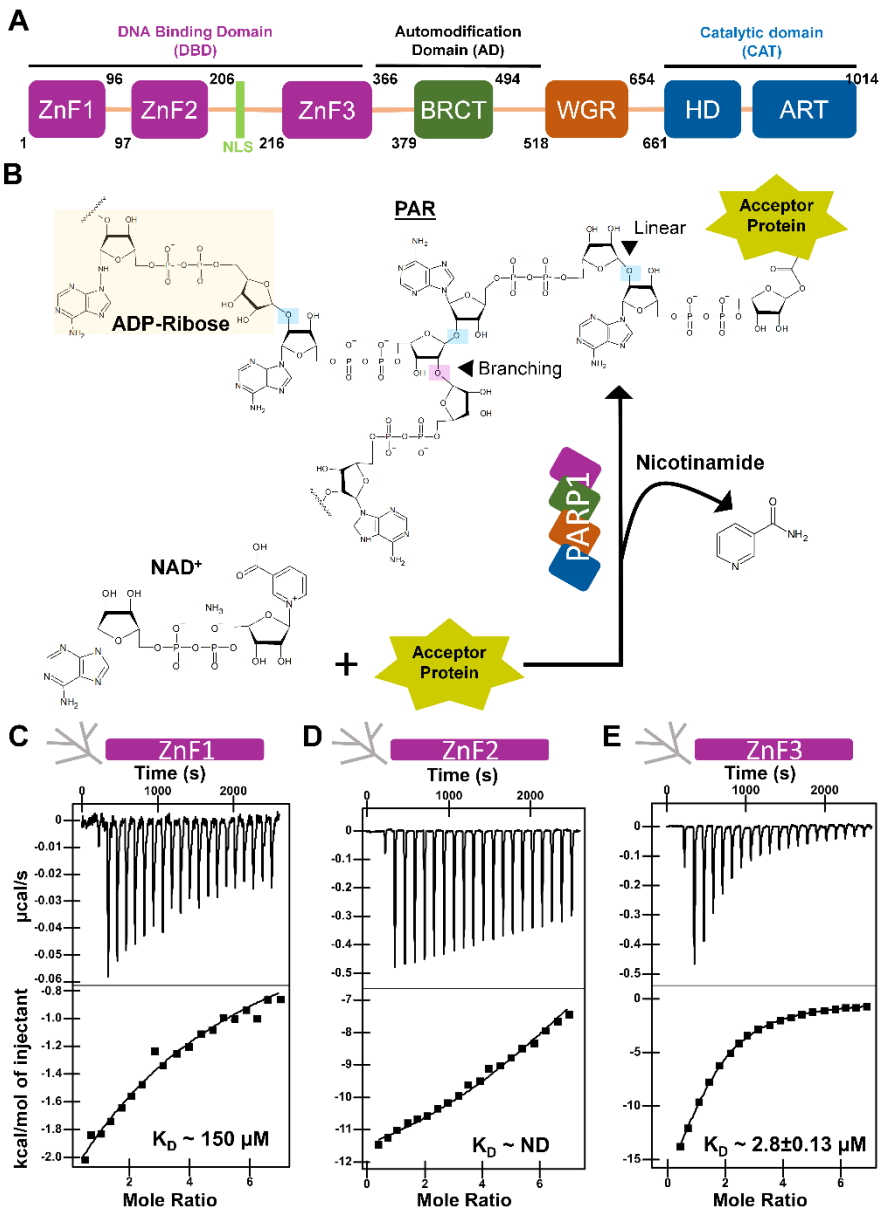

Figure 1. Domain architecture of PARP1 and PAR binding studies. Schematic representation of (A) domain architecture of $PARP1_{FL}$ and (B) Poly(ADP-ribosyl)ation (PARylation) reaction catalyzed by PARP1. ITC plots of PAR titrated with (C) ZnF1, (D) ZnF2 and (E) ZnF3. ND stands for 'Not determined'. In the figures 1 to 2 branched tree on the top of ITC panel indicates the PAR.

WGR and BRCT domains of PARP1 bind to PAR

We performed ITC binding studies of PAR with BRCT and WGR domain of PARP1. Surprisingly, the WGR domain didn't only bind to PAR but showed a high binding affinity with a K_D of 5.1 µM (Figure 2A). BRCT domain's binding affinity is similar to WGR's (K_D ~4.2 µM) (Figure 2B). On the contrary, neither WGR nor BRCT domains recognize ADPr, the repeating unit of PAR (Supplementary Figure 2A-B).

Calorimetric binding studies of combination of PARP1 domains to PAR

To determine the cooperativity in PARP1 domains to bind to PAR, we performed binding studies. Our ITC data show that ZnF1-BRCT binds to PAR with several folds

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more binding affinity compared to individual domains, i.e., K_D ~0.6 μM (Figure 2C). On the other hand, ZnF1-BRCT also did not bind to ADPr (Supplementary Figure 2C). WGR-CAT showed a K_D of 2.7 μM for PAR (Supplementary Figure 3A) whereas, the catalytic domain (CAT) showed a weaker affinity (K_D ~ 39.1 μM) (Supplementary Figure 3B).

Calorimetric binding studies of PARP1 domains to PARylated proteins

Our ITC based binding studies showed that the WGR and BRCT domains of PARP1 bind to PARylated PARP2 with a K_D of 2.9 and 2.4 μM, respectively (Figure 2 D-E). Further, titration of PARylated PARP2 against quadruple ZnF1-BRCT domain of PARP1 showed a K_D of 0.2 μM (Figure 2F). While these domains alone did not bind to un-PARylated PARP2 (Supplementary Figure 4).

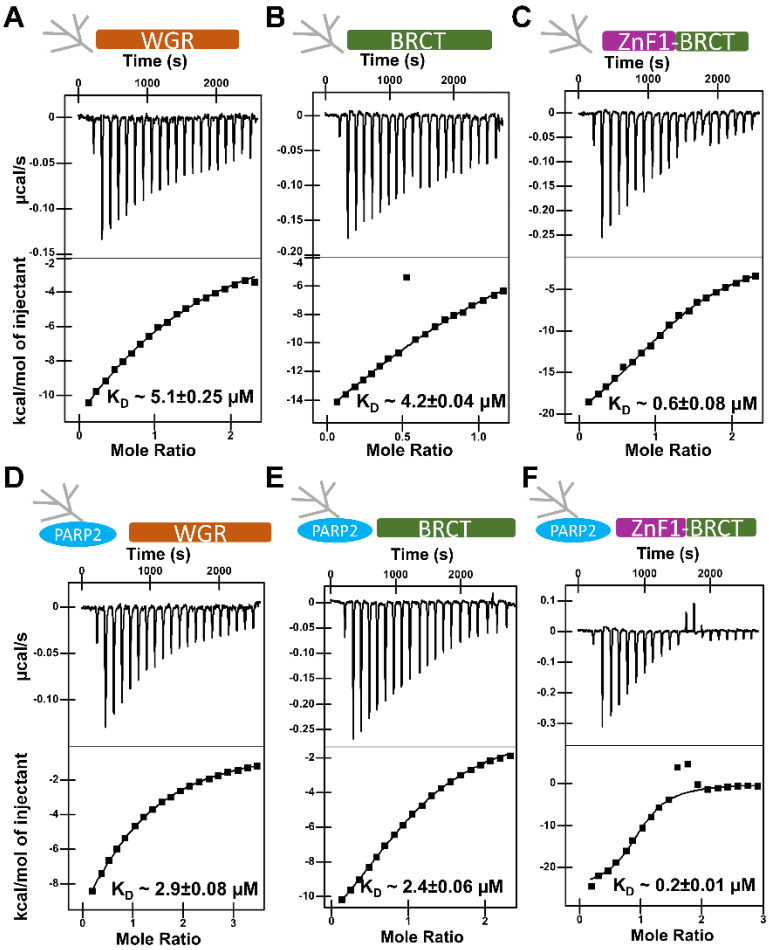

Figure 2. ITC binding studies of PAR with (A) WGR (B) BRCT (C) ZnF1-BRCT, and PARylated PARP2 with (D) WGR (E) BRCT (F) ZnF1-BRCT.

Calorimetric studies of the ssDNA binding specificity of the PARP1 domains

The calorimetric binding studies of individual domains of PARP1 with a single strand DNA (ssDNA) and a ssDNA with 5'-phosphate (5'P-ssDNA) showed that among zinc fingers, ZnF2 binds to both the DNAs with highest and almost a similar affinity (K_D ~4.3 μM), whereas ZnF1 exhibited a K_D of 6.3 μM for 5'P-ssDNA and a K_D of 5.4 μM for ssDNA. (Figure 3A-D). ZnF3 binds neither ssDNA nor 5'P-ssDNA (Figure 3E-F).

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Whereas WGR binds neither DSB nor SSB DNA, and also does not exhibit binding towards 5'P-ssDNA and ssDNA (Supplementary Figure 5).

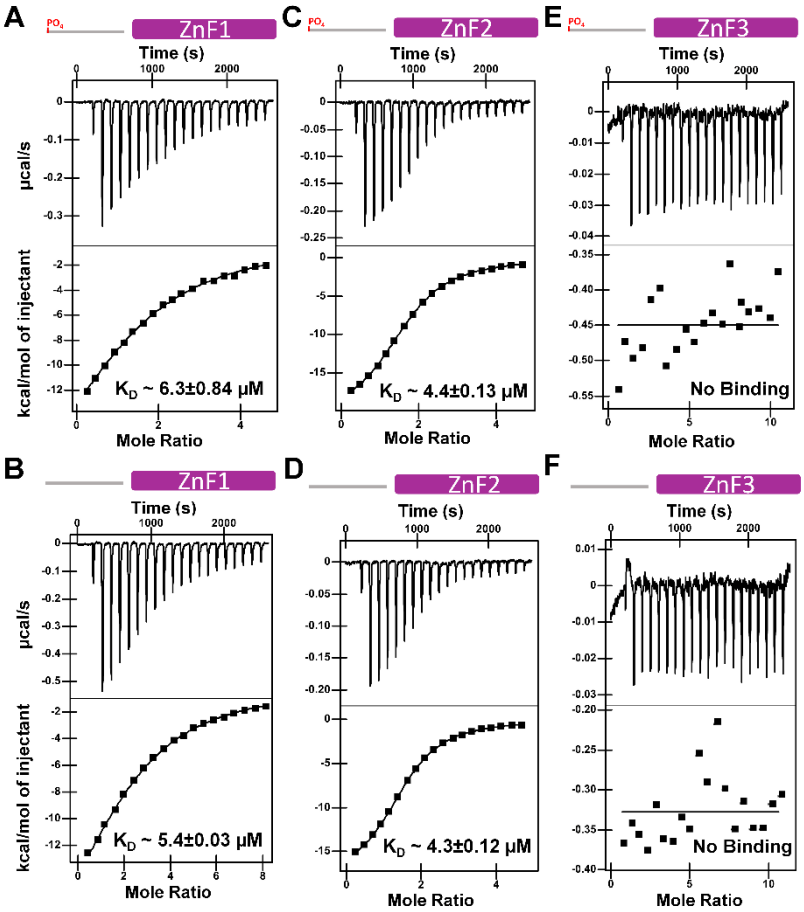

Figure 3. ITC binding studies between single stranded DNAs, with and without 5'P, and zinc finger domains of PARP1. ITC plot of ZnF1 binding to (A) 5'P-ssDNA and (B) ssDNA; ZnF2 binding to (C) 5'P-ssDNA and (D) ssDNA; and ZnF3 binding to (E) 5'P-ssDNA and (F) ssDNA.

Competition binding studies of PAR and different DNAs to ZnF1-BRCT quadruple domains of PARP1

We carried out fluorescence polarization (FP) based competition binding studies to investigate whether PAR can compete with different damaged DNAs to bind ZnF1-BRCT quadruple domains. Our FP binding studies shown that ZnF1-BRCT binds to DSB, SSB and 5'P-ssDNA with a K_D of 37.8 nM, 43.8 nM and 83.6 nM, respectively (Figure 4 A-C). ITC binding studies underestimated the binding affinity between ZnF1-BRCT and above DNAs, but relative binding affinities are highly correlated (Supplementary Figure 6 and Figure 4). Competition FP assays were performed to evaluate the displacement of the fluorophore labelled bound DNA probe by PAR. The molar equilibrium dissociation constant (*Ki*) of the unlabeled competitor PAR was determined to be 104.8 nM, 47.3 nM and 50.3 nM, respectively for DSB, SSB and 5'P-ssDNA bound to ZnF1-BRCT (Figure 5 A-C).

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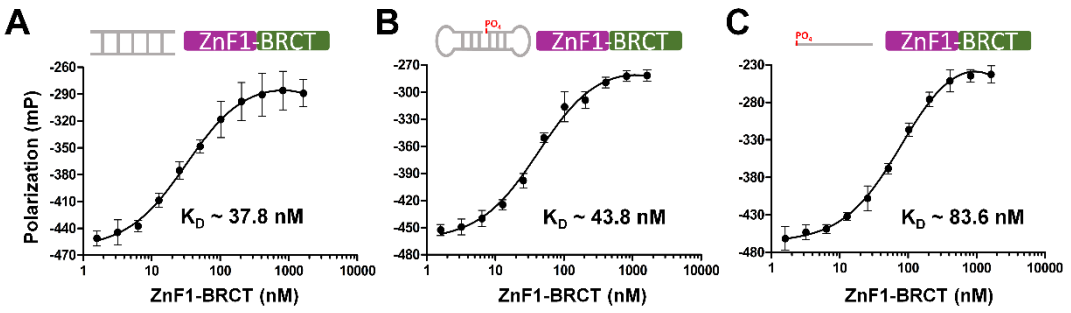

Figure 4. Fluorescence polarization binding studies of ZnF1-BRCT with (A) DSB DNA, (B) SSB DNA and (C) 5'P-ssSNA.

Competition binding studies of PAR and different DNAs to $PARP1_{FL}$

Our FP binding studies shown that the $PARP1_{FL}$ binds to DSB, SSB and 5'P-ssDNA with a K_D of 9.9 nM, 33.6 nM and 29.6 nM respectively (Supplementary Figure 7). The *Ki* of the competitor PAR was determined to be 68.1 nM, 14.6 nM and 6.4 nM, respectively, for DSB, SSB and 5'P-ssDNA bound to $PARP1_{FL}$ (Figure 5 D-F).

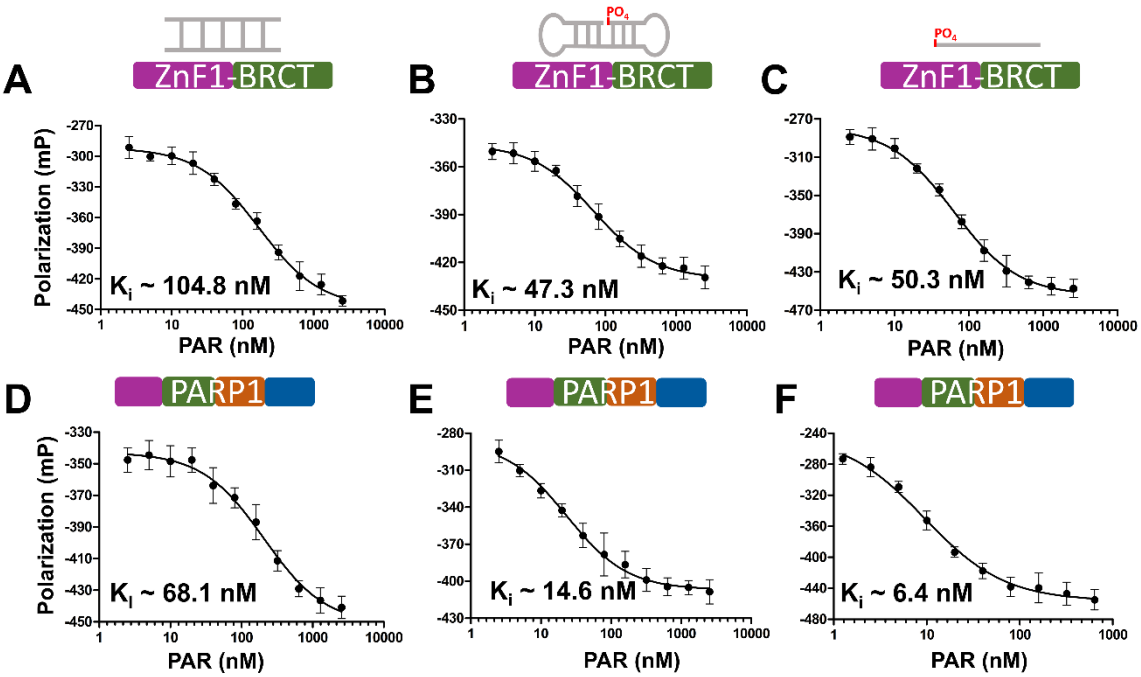

Figure 5. Fluorescence polarization based competition binding study. Competition binding study of unlabeled PAR for ZnF1-BRCT domains of PARP1 against fluorophore labelled (A) DSB DNA, (B) SSB DNA and (C) 5'P-ssDNA. Competition binding study of unlabeled PAR for $PARP1_{FL}$ against fluorophore labelled (D) DSB DNA, (E) SSB DNA and (F) 5'P-ssDNA.

Effect of PAR on catalytic activity of PARP1

Our PNC1-OPT based enzyme activity assay shows 4-fold increase in PARylation activity of $PARP1_{FL}$ in the presence of PAR, compared to its absence after 30 minutes of incubation (Figure 6A).

Effect of PAR on DNA dependent stimulation of PARP1's catalytic activity

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Our activity assay shows that ssDNA and SSB DNA independently stimulate $PARP1_{FL}$ activity by 10 and 12 folds, respectively (Figure 6). Though PAR stimulates the catalytic activity of the $PARP1_{FL}$, the PAR dependent stimulation is significantly less (3-3.5 times) compared to ssDNA and SSB DNA dependent activation of the $PARP1_{FL}$ (Figure 6). At the same time, PAR decelerates both the ssDNA and SSB DNA dependent stimulation of the PARP1's catalytic activity by 22% and 18%, respectively (Figure 6B-C).

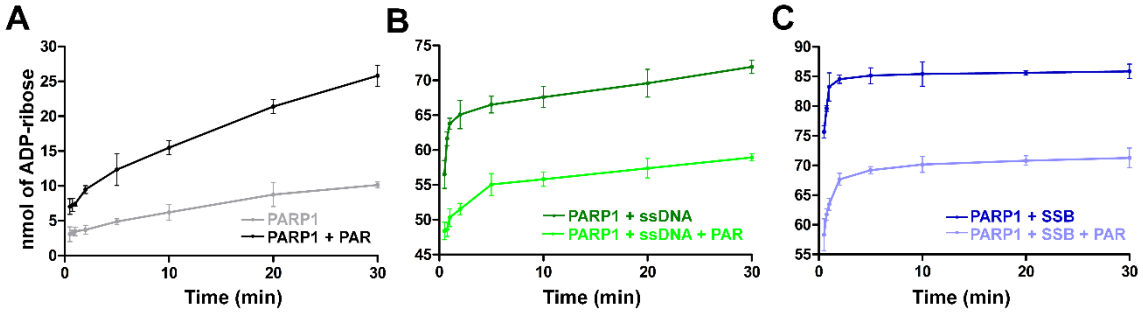

Figure 6. Colorimetry assay of PARP1 self-PARylation catalytic activity. Effect of PAR on PARP1 activity in (A) absence of DNA, and presence of (B) ssDNA and (C) SSB DNA. Error bars indicates the standard deviation of the three independent experiments.

DISCUSSION

PARP1 has been known for over six decades, yet its regulatory mechanism and binding partners have not been completely understood. In addition to till known regulators of PARP1, here we report that PARP1 is also regulated by its own catalytic product, PAR. Our studies reveal that PARP1 differentially recognize their binding partners, i.e., PAR and DNA, via dedicated domains. The investigation reveals ZnF3, BRCT and WGR domains of PARP1 as a bonafide reader of PAR, whereas ZnF1 and ZnF2 exhibited least to no affinity for the same (Figure 1C-E and Figure 2A-C). Contrarily, ZnF2 and ZnF1 have specific binding towards DNA, whereas ZnF3, BRCT and WGR domains do not bind to DNA (Supplementary Figure 5, 8 and 9). CAT domain, on other hand, show a weak affinity for PAR. These analyses suggest that, among reader domains, DNA reader domains are contiguous and are at the N-terminal of PARP1, similarly, PAR reader domains are also contiguous, but they are in the central region of the protein. The binding partners, other than damaged DNA and a few proteins (55), which could regulate the DNA binding and catalytic activities of PARP1 haven't been explored extensively. This study identifies the PAR reader domains of PARP1, and delineates the role of PAR in displacement of DNA bound to PARP1 and as a modulator of the catalytic activity of PARP1.

ZnF domains of PARP1 differentially recognize PAR and DNAs

ZnF domains of PARP1 have been known to recognize DNA breaks, where ZnF1 and ZnF2 together recognize SSB DNA, and ZnF1 along with ZnF3 recognize DSB DNA (16, 17). Additionally, ZnF3 has been reported to be involved in chromatin compaction and facilitates the inter-domain contacts for PARP1 activation rather than being directly involved in DNA binding (56). Our studies suggest that only ZnF1 and ZnF2 are directly involved in DNA binding, and not ZnF3 (Supplementary Figure 8). ZnF3, on the other hand, binds strongly to PAR (Figure 1E). Structural comparison of zinc fingers shows that ZnF3 is significantly different from ZnF1 and ZnF2 as it could not

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be superimposed on either of the two ZnFs, whereas ZnF1 and ZnF2 share a structural similarity with a RMSD of 0.6 Å for Cα atoms (Supplementary Figure 10). Besides, canonical Zinc fingers, ZnF1 and ZnF2, have weak to no affinity for PAR (Figure 1C-D). These data show that zinc fingers of PARP1 have different preference for their binding partners.

Recognition of single strand DNA by PARP1 and its implications

Recently it's been reported that PARP1 activity is greatly elevated by tens of kilobases behind DNA replication forks where un-ligated Okazaki fragment intermediates and DNA single strand gaps are observed in high frequency (57, 58). In addition, PARP1 is reported to be involved in replication fork remodelling when the DNA lesions are ahead of them (59, 60). In this study, for the first time, we have shown that PARP1, through its ZnF1 and ZnF2 domains, binds to ssDNA with a high affinity as that of SSB and DSB DNA (Figure 3, Supplementary Figure 8) which supports the role of PARP1 in above phenomenon. Though, ssDNA is a structural analog of PAR (Supplementary Figure 1), both are recognized by different domains of PARP1.

BRCT domain binds to PAR but not DNA

BRCT domains of BRCA1 and MDC1 have been reported to recognize phosphorylated serine (Sph) while BRCT domains of XRCC1 and ligase 4 have been shown to recognize PAR, in micromolar binding affinity similar to BRCT domain of PARP1, as well as its monomeric unit, ADPr (29). Surprisingly, our ITC binding studies show that BRCT and other PAR binding domains of PARP1 are involved in recognizing PAR polymer but fails to bind ADPr monomer (Figure 1E; Figure 2A-B and Supplementary Figure 2) and also phosphorylated H2A.X peptide (data not shown). This suggests that BRCT and other PAR binding domains of PARP1 have a different PAR recognition mechanism compared to PAR binding domains of other proteins. Additionally, BRCT domain of PARP1 is also different from BRCT domain of other proteins which bind to Sph peptide. Our binding studies recapitulated previous reports that BRCT domain showed no binding to different DNAs (Supplementary Figure 9).

WGR domain binds to PAR and assists the DSB DNA recognition

The WGR and BRCT domains of PARP1 have not been functionally characterized. On the other hand, PARP2 WGR has been shown to be involved in recognition of DNA (32). Our study shows that PARP1 WGR does not bind to any of the tested DNAs (Supplementary Figure 5). Here, for the first time we show that, PARP1 WGR domain recognizes PAR with a binding affinity on par with PARP1 BRCT. Thus, our study opens a window for the characterization of WGR domain of PARP2 and PARP3 for recognition of PAR, and its possible implications in DNA repair and epigenetic processes.

Binding affinity of $PARP1_{FL}$ to DSB DNA is more than SSB DNA (Supplementary Figure 7A, B). In addition, binding affinity of $PARP\text{-}1_{FL}$ is more than ZnF1-BRCT quadruple domain (Figure 4 and Supplementary Figure 7) which lacks WGR domain. These suggest that though WGR domain, standalone, does not bind to DSB DNA, it could be required for DNA dependent catalytic activity stimulation and may contribute to DNA binding in association with other domains of PARP1 (Figure 4 A and Supplementary Figure 7 A). This study corroborates with the crystal structure of the ZnF1, ZnF3 and WGR-CAT domains of PARP1 assembled on the DSB DNA (PDB ID: 4DQY), where WGR domain has a network of interactions with the 5' terminal sequence of the DNA (16). In case of SSB, binding affinity of ZnF1-BRCT and $PARP_{FL}$

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is almost same, indicating that WGR domain may not contribute for the recognition of SSB DNA (Figure 4 B and Supplementary Figure 7B).

PARP1 reader domains exhibit higher binding affinity for PARylated protein compared PAR

The BRCT and WGR domains of PARP1 not only bind to free PAR but also to PARylayed protein with higher affinities (Figure 2A,B,D,E) implicating that the recognition of PAR by PARP1 is not a random event but might have a biological significance. The higher binding affinity for PAR and PARylated protein by ZnF1-BRCT compared to individual domains (ZnF3 and BRCT) shows that these domains show synergism for PAR recognition (Figure 1E and Figure 2B,C,E,F).

PAR allosterically regulate DNA binding to PARP1, and ZnF3-BRCT-WGR triple domains might play a major role in displacement of PARP1 from DNA in presence of PAR

Since the binding of DNA to $PARP1_{FL}$ is tighter than ZnF1-BRCT quadruple domains of PARP1 (Figure 4 and Supplementary Figure 7), therefore, expectedly, Ki values for displacement of DNA from the $PARP1_{FL}$-DNA complex by PAR should be more for displacement of DNA from ZnF1-BRCT-DNA complex. In contrast, higher concentrations of PAR were required for displacement of DNA from ZnF1-BRCT than $PARP1_{FL}$ (Figure 5). Major difference between ZnF1-BRCT and $PARP1_{FL}$ is $PARP1_{FL}$ has an additional PAR binding domain WGR. Although WGR domain is not involved in DNA binding (Supplementary Figure 5), but it could negatively affect the damaged DNA binding to ZnFs, allosterically, through binding to PAR. Thus, PAR binding to ZnF3-BRCT-WGR triple domains could induce global conformational changes in the PARP1 making it incompetent to recognize DNA by ZnF1 and ZnF2 leading to dissociation of PARP1-DNA complex (Figure 7). Adaptation of compact structural conformation from the extended beads-on-string conformation by PARP1 upon DNA binding (16), supports our hypothesis of PAR-induced alternative global conformation in PARP1, though yet to be studied. It is noteworthy that certain PARP1 inhibitors (PARPi) bind to the catalytic domain of PARP1 and allosterically enhance either release or retention of PARP1 on the DNA (61–63). PAR may function analogously to the PARPi(s), which release the PARP1 from the bound DNA.

It has already been reported that self-PARylation of PARP1 leads to release of DNA from the PARP1 (64). Recently, through EMSA studies, $PARP1_{FL}$ has been shown to bind to PAR with a nanomolar binding affinity (65). Binding of PAR or PARylated proteins by the PAR-reader domains of PARP1 could lead to DNA dislodging from PARP1 itself. These phenomena are also supported by our studies which show that PAR binding domains of PARP1 exhibited higher affinity towards PARylated protein than PAR (Figure 2) and binding of PAR leads to DNA dissociation from PARP1 (Figure 5).

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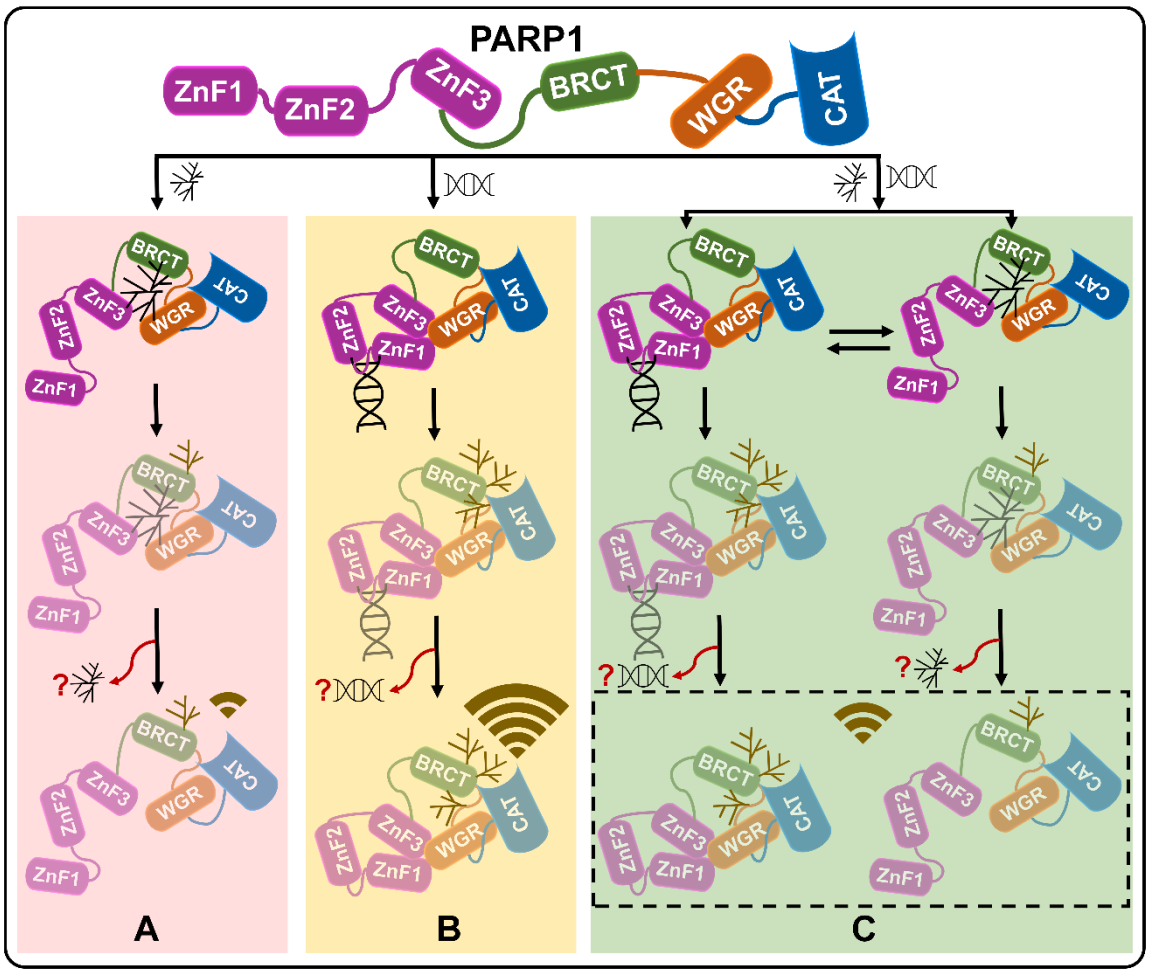

Figure 7. Schematic representation of proposed allosteric regulation of PARP1 by PAR and DNA. (A) Binding of PAR to ZnF3-WGR domains may lead to spatial reorganization of PARP1 domains to get into certain conformation that stimulates the catalytic activity of PARP1. (B) DNA binding induces conformational changes in the protein that leads to its maximal activation. (C) Binding of PAR and DNA to PARP1 might be non-competitive reversible that may lead to two different conformational outcomes, resulting in differential catalytic stimulation, which could be an average of PAR- and DNA-dependent catalytic stimulation of the PARP1. The displacement of DNA by the PAR from the DNA-PARP1 complex eventually retards the DNA dependent stimulation but at the same time PAR binding itself stimulate PARP1 activity, therefore the activation is modulated by cross talk between DNA and PAR binding to PARP1.

PAR is a partial agonist of the PARP1

Compared to DNA recognition, PAR is not only differentially recognized by PARP1 but also stimulates its enzymatic activity (Figure 6A). However, stimulation of activity is lesser than DNA dependent stimulation which suggests that PAR can be a partial agonist of PARP1, and difference in stimulation of activity supports that these polymers altogether induce different but catalytically competent global conformations of PARP1. This property of the PARP1 suggests that DNA independent stimulation of enzymatic activity may be required for PARylation of proteins involved in nuclear processes other than DNA repair (12).

PAR retards the DNA dependent stimulation of PARP1's catalytic activity

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PAR and DNA independently stimulate the PARP1 catalytic activity (Figure 6), suggesting that PAR and DNA could synergistically stimulate the PARP1 activity. Surprisingly, instead of enhancing, PAR decelerates DNA dependent enzymatic activity of PARP1 (Figure 6B-C). For PAR being an agonist, the magnitude of retardation of DNA dependent PARP1 stimulation by PAR might be much more than what we observed in our experiments. In nutshell, PAR acts as both partial allosteric stimulator and partial allosteric inhibitor (partial antagonist) of PARP1 (Figure 7), and at the same time PAR may itself put a check on the amount of PAR which could be formed upon stimulation. Given that the PAR length and branching have specific functions in cellular physiology and stress response (22), our study suggests that PAR dependent regulation of PARP1 activity might have additional biological implications as well. Recently, it has been reported that protein factor, HPF1, stimulates the DNA-dependent and independent self-PARylation of PARP1 and PARP2 as well as the trans-PARylation of histones in the complex with nucleosome (66). Likewise, PAR stimulates the self-PARylation of PARP1 but, unlike HPF1, it hampers the DNA-dependent self-PARylation of PARP1. However, the effect of PAR or PARylated proteins on trans-PARylation activity of PARP1 on histones, other proteins or nucleic acid substrates of PARP1 needs to be addressed. Like Histone H4, PAR also stimulates PARP1's catalytic activity through binding to its C-terminal domains (67). However, it's not known whether H4 hampers DNA dependent stimulation of PARP1's activity like PAR.

PARP1 is both writer and reader of PAR code

Histone writers, erasers, and readers – the protein machineries that add, remove, or recognize post-translational modifications (PTMs)also known as histone or PTM codes, respectively – have become a cornerstone in understanding the role of epigenetics in transcription, DNA replication & repair and splicing. PARylation, is an epigenetic PTM mark, and PAR code is involved in above process through chromatin remodeling, heterochromatin reorganization, histones exchange, etc. (68, 69). Several DNA methyltransferases, histone lysine methyl- and acetyl-transferases have both writer and reader domains of corresponding epigenetic mark, and both reading and writing of the marks direct a particular epigenetic outcome (70–72). Similarly, our studies establish that besides a PAR code writer domain, PARP1 also contains dedicated domains for reading PAR code. Therefore, our studies set the platform to understand the cross talk between reading and writing of a PAR code by the PARP1 on epigenetic gene regulation and/or DNA replication and repair.

Based on our findings we propose the allosteric regulation of PARP1 by its own catalytic product, PAR. Further, structural studies of PAR with PAR binding domains of PARP1 (individual or in combination) and $PARP1_{FL}$ would reveal mechanistic insights, and that could also help in designing of allosteric inhibitors or stimulators of PARP1. Moreover, these inhibitors would be very specific, since most of the available inhibitors (FDA approved or in clinical trials) target the NAD+ binding site, which could lead to non-specific targeting/ inhibition of other proteins of PARP family. Also, PAR recognition could be a kind of feedback regulation, which regulates the extent of PARylation by PARP1. This study also suggests the alternative pathway of stimulation of PARP1's catalytic activity in the nucleus of a cell independent of DNA damage signaling.

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