Crystal structures and functional analysis of the ZnF5-WWE1-WWE2 region of PARP13/ZAP define a distinctive mode of engaging poly(ADP-ribose)

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SUMMARY

PARP13/ZAP (zinc-finger antiviral protein) acts against multiple viruses by promoting degradation of viral mRNA. PARP13 has four N-terminal zinc (Zn) fingers that bind CG-rich nucleotide sequences, a C-terminal ADP ribosyltransferase fold, and a central region with a fifth Zn finger and tandem WWE domains. The central PARP13 region, ZnF5-WWE1-WWE2,
is implicated in binding poly(ADP-ribose); however, there are limited insights into its structure and function. We present crystal structures of ZnF5-WWE1-WWE2 from mouse PARP13 in complex with ADP-ribose and in complex with ATP. The crystal structures and binding studies demonstrate that WWE2 interacts with ADP-ribose and ATP, whereas WWE1 does not have a functional binding site. Binding studies with poly(ADP-ribose) ligands indicate that WWE2 serves as an anchor for preferential binding to the terminal end of poly(ADP-ribose) chains. The composite ZnF5-WWE1-WWE2 structure forms an extended surface to engage ADP-ribose chains, representing a distinctive mode of recognition that provides a framework for investigating the impact of poly(ADP-ribose) on PARP13 function.

**In brief**

Kuttiyatveetil et al. report a structural and biochemical analysis of the poly(ADP-ribose) binding region of PARP13/ZAP, an RNA-binding protein involved in the antiviral response. X-ray structures and SAXS analysis, together with binding studies using ADP-ribose ligands of defined length, describe an unconventional mode of engaging the poly(ADP-ribose) posttranslational modification.

**Graphical Abstract**

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INTRODUCTION

PARP13 belongs to the ADP-ribosyltransferase (ART) protein family that uses NAD$^+$ to create ADP-ribose (ADPr) posttranslational modifications (Hottiger et al., 2010; Lüscher et al., 2018, 2021; Vyas et al., 2014). Although certain family members produce poly(ADPr) modifications, most family members produce mono(ADPr) modifications, whereas PARP13 lacks ART activity entirely (Hottiger et al., 2010; Karlberg et al., 2015; Kleine et al., 2008; Lüscher et al., 2018; Vyas et al., 2014). Poly(ADPr) and ADPr modifications act in a variety of manners to coordinate cellular processes, such as the DNA damage response, gene regulation, cellular signaling, and the antiviral response.

PARP13 was first known as zinc-finger antiviral protein (ZAP) because of the presence of Zn fingers and activity against retroviral RNA (Gao et al., 2002). The antiviral response is accomplished through PARP13 recognition of cytoplasmic mRNA, which recruits RNA degradation machinery through multiple mechanisms to eliminate target viral mRNA (Guo et al., 2007; Zhu and Gao, 2008). PARP13 overexpression is associated with replication inhibition of viruses, such as filovirus, alphaviruses, and numerous others (Bick et al., 2003; Liu et al., 2015; Mao et al., 2013; Müller et al., 2007). Human PARP13 (hP13)/ZAP has four splice variants of varying lengths, with the major difference being that the two shortest isoforms lack the ART domain entirely (Li et al., 2019). Although all four isoforms exhibit similar antiviral activities against most virus groups, the longer isoforms containing the ART domain exhibit greater antiviral potential against alphaviruses and hepatitis B virus (HBV).

The N-terminal domain of PARP13 has four CCCH-type Zn-finger motifs that collectively bind CpG-rich RNA sequences (Takata et al., 2017). Recently, crystal structures of human and mouse PARP13 (mP13) N-terminal Zn fingers bound to RNA have revealed key elements of RNA recognition (Luo et al., 2020; Meagher et al., 2019). For example, the CpG dinucleotide is specifically recognized by the second Zn finger, whereas the other Zn-finger contacts with single-strand RNA are not sequence specific. It is postulated that multiple PARP13 molecules will bind to regions of viral RNA rich in CpG dinucleotides, and the assembly of multiple PARP13s will then act as a signal to recruit the mRNA destruction complex (Luo et al., 2020). However, the molecular mechanism for PARP13 signaling of RNA recognition is not understood and will likely require further insights into the structure of PARP13.

Compared with the N-terminal Zn fingers and the C-terminal ART domain, much less is known about the structure and function of the central region of PARP13, which is present in all four isoforms. This central region contains ~270 residues of low-complexity sequence that are likely unfolded, followed by ~220 residues just before the ART domain, which are predicted by sequence to contain a Zn finger and two WWE domains. WWE domains (named for conserved residues Trp-Trp-Glu) are protein modules known to form protein-protein interactions or to bind to poly(ADPr) (Wang et al., 2012). Indeed, PARP13 has been identified as a poly(ADPr)-binding protein in proteomic analyses (Dasovich et al., 2021; Kliza et al., 2021). Furthermore, a recent study demonstrated that poly(ADPr) binding modulates the antiviral activity of PARP13/ZAP (Xue et al., 2022).
We undertook a structural and functional analysis of the central PARP13 region predicted to contain folded structures (ZnF5-WWE1-WWE2), because none of these features had been confirmed. It was also unclear whether the predicted domains formed a single globular unit or a collection of protein modules. Moreover, the properties of tandem WWe domains were intriguing, because the best-studied WWe domain that is known to interact with poly(ADPr), that of RNF-146, utilizes a single WWe domain to bind the ribose-ribose linkage of poly(ADPr) (Wang et al., 2012). Here, we report the crystal structure of the ZnF5-WWE1-WWE2 region of mP13 in complex with ADPr and in complex with ATP, and we compare our structure with the recently published structure of the same region of hP13 (Xue et al., 2022). Based on binding studies using defined poly(ADPr) ligands, we propose a model for ZnF5-WWE1-WWE2 engagement of ADPr chains starting from an anchor point on the terminal ADPr unit.

RESULTS

Production and crystallization of ZnF5-WWE1-WWE2

Several hP13 domain boundaries were tested for soluble protein production, including multiple constructs of ZnF5-WWE1-WWE2, constructs for individual domains (ZnF5, WWe1, or WWe2), and constructs for tandem WWe’s (WWe1-WWe2). Three constructs for ZnF5-WWe1-WWe2 (residues 468–699, 487–699, and 507–699) produced soluble protein, and the construct coding for residues 507–699 was selected to represent ZnF5-WWe1-WWe2 of hP13, because it included all predicted domains in a minimal sequence (Figure 1A). None of the constructs designed to produce individual domains or domain combinations produced soluble protein that would permit further studies. Together, the expression studies indicated that the domains were best produced in combination, suggesting that they form a globular assembly. Similar observations were made by Xue et al., (2022) studying the same region of hP13. We were unable to crystallize hP13 ZnF5-WWe1-WWe2 using several constructs in the presence or absence of ligands. We also cloned and produced the same ZnF5-WWe1-WWe2 region from mP13 (residues 476–673), which shares 60% identity with hP13 over this region. mP13 ZnF5-WWe1-WWe2 yielded diffraction quality crystals in the presence of ATP and in the presence of ADPr. The crystal structure was determined by single-wavelength anomalous diffraction (SAD) using selenomethionine-containing protein, and the SAD structure was used for molecular replacement determination of ZnF5-WWe1-WWe2 complex structures with resolutions of 2.2 Å for the ADPr complex and 2.2 Å for the ATP complex (crystallographic statistics are in Table 1).

Overview of the mP13 ZnF5-WWe1-WWe2 crystal structure

mP13 ZnF5-WWe1-WWe2 crystallized with two nearly identical molecules in the asymmetric unit (Figure 1B, red and green molecules). The two molecules of mP13 ZnF5-WWe1-WWe2 form a 2-fold symmetric dimer (Figure 1B), with an extensive buried surface area of over 5,000 Å². Each molecule contains two WWe folds, although with key differences distinguishing WWe1 and WWe2. Most notably, only WWe2 showed evidence of bound ADPr/ATP, and WWe1 lacked a binding pocket. The ZnF5 residues anticipated to form a CCCH-type Zn finger (Zn-coordinating residues: C488, C496, C501, and H505) were only partially modeled because of a lack of electron density for certain
regions, presumably because of disorder. The modeled region of ZnF5 is positioned adjacent to WWE1. In each of the two molecules, ZnF5-WWE1 is linked to WWE2 by a 15-residue, extended linker (one monomer is shown in Figure 1C). Following WWE2 is a small helix that would in turn lead to the ART domain in the longer isoforms of PARP13.

We noted that WWE1 and WWE2 domains from different molecules form a large surface area burying over 2,000 Å² (e.g., green WWE1 with red WWE2 in Figure 1B), whereas WWE domains from the same chain form a smaller contact area less than 800 Å² (see Figure 1C). We also noted that a simple crossing over between polypeptides in the extended linker near the axis of 2-fold symmetry allowed us to model the more extensive WWE1/WWE2 interactions as arising from the same polypeptide (see crossing over in Figure 1D and subsequent model in Figure 1E). The resulting configuration of the two WWE domains is substantially more compact than the extended configuration (Figure 1E versus 1C, respectively). Notably, this compact arrangement of WWE domains agrees with the monomeric arrangement of WWE domains observed in the crystal structure of hP13 ZnF5-WWE1-WWE2 (Xue et al., 2022) (Figure 2A).

To address the multimeric state of ZnF5-WWE1-WWE2 in solution, we used SEC-MALS (size exclusion chromatography/multi-angle light scattering) analysis of mP13 and hP13 (Figure 2B). The elution profile for mP13 had two peaks: a major peak at ~16.5 mL representing 96% of the scattering mass and estimated at 25.7 kDa ± 0.5% and a minor peak at ~15.3 mL representing ~4% of the scattering mass and estimated at 49 kDa ± 1.7%. These mass values best estimate a monomer (96%) and dimer (~4%) of mP13 ZnF5-WWE1-WWE2 (theoretical mass of 23.6 kDa). The elution profile for hP13 also exhibited two peaks. The larger peak at ~16.7 mL represented 98% of the scattering mass and was estimated at 25.1 kDa ± 0.4%. The scattering signal from the smaller hP13 peak was not sufficient to provide a mass estimate, but its location appears similar to the dimer peak observed in the mP13 sample. Therefore, both mP13 and hP13 exist primarily as monomers in solution, with some evidence for a small population of dimers.

We further analyzed the solution properties of mP13 and hP13 using SEC coupled with small-angle X-ray scattering (SEC-SAXS) (Figures S1A–S1F), which can provide molecular mass and size information, as well as the overall solution conformation. Buffer-subtracted SAXS profiles were processed to obtain the structural parameters radius of gyration (Rg), maximum particle dimension (Dmax), and molecular mass (mP13: Rg, 20.9 Å; Dmax, ~72 Å; molecular mass, 21.3 kDa; hP13: Rg, 20.2 Å; Dmax, ~65 Å; molecular mass, 19.8 kDa; Figure S1C). These measurements for mP13 and hP13 are consistent with a monomeric conformation of ZnF-WWE1-WWE2. Ab initio modeling of the solution scattering data also supported the monomeric, compact assembly for ZnF5-WWE1-WWE2 (Figures S2A–S2D). Thus, despite the crystallographic evidence for a dimer of mP13 ZnF5-WWE1-WWE2 (Figures S2A–S2D). Thus, despite the crystallographic evidence for a dimer of mP13 ZnF5-WWE1-WWE2, the solution-based structural analysis support a monomeric form.

Comparison of the mouse and human crystal structures of ZnF5-WWE1-WWE2 highlights the relationship between the partial ZnF5 structure and the folded ZnF5 structure (Figure 2A). Zn-coordinating residue H505/H531 (mP13/hP13 numbering) is in a roughly similar position in both structures, held near one end of WWE1. In mP13, residues C488 and
C496 are located on two anti-parallel β strands on the side of WWE1 and positioned away from H505; C501 was located in the disordered region that was not modeled. In hP13, the corresponding residues (C513, C521, and C527) are clustered around H531 to form the Zn(II) binding site. A transition between the two models would require a re-structuring of the β strands bearing C488/C496 to form a helical structure. Zn content analysis of hP13 and mP13 ZnF5-WWE1-WWE2 indicated that these proteins co-purified with stoichiometric amounts of Zn (Figure 2C), suggesting that the formation of the dimer in the crystal lattice might have driven the destabilization of ZnF5 and the loss of bound Zn(II). Indeed, the β strand bearing C488 interacts with the extended linker region that forms the majority of the dimer interface (Figures 1B and 1C), which could be the basis for disruption of ZnF5 in the mP13 structure. Notably, mP13 crystals soaked with up to 1 mM Zn chloride or co-crystallized with equimolar amounts of Zn chloride did not lead to Zn-bound structures.

Thus, the mP13 crystal structure captured the essential attributes of the compact conformation of ZnF5-WWE1-WWE2, albeit arising from two different protein chains. We thus view the crystal structure as representative of the monomeric solution state of ZnF5-WWE1-WWE2 and appropriate for analyzing ADPr/ATP ligand interactions.

Variations in the WWE domain fold

Although mP13 ZnF5-WWE1-WWE2 was crystallized in the presence of 1 mM ADPr or 1 mM ATP, ligand was observed bound only to WWE2 (Figures 3A and 3B). There was no evidence for ligand interaction with WWE1, despite the domain being accessible within the crystal lattice. The binding pocket of WWE2 is formed at one end of a barrel-shaped fold composed of six β strands and one α helix (Figures 3A and 3B). The loops connecting the secondary structure elements and the central cavity at one end of the barrel contribute the major interacting residues (Figure 3C), centered around W585 that engages one face of the adenine base (W611 in hP13). Specific contacts are also made with the adenosine ribose and two phosphate groups of ADPr/ATP; however, there are no protein atoms with 4 Å of the terminal γ-phosphate of ATP. The structure of hP13 in complex with ADPr provided similar insights into the ligand binding pocket of WWE2 (Xue et al., 2022).

A comparison of the WWE1 and WWE2 domains at the sequence and structural level provides a clear basis for the observed differences in binding to ligand (Figures 3D, 3E, S3A, and S3B). WWE1 and WWE2 share the same barrel-shaped fold, but the connecting loops are much shorter in WWE1. Most notably, one loop present in WWE2 (residues 594–608) is over 10 residues shorter in WWE1, entirely removing a major portion of the binding surface (Figure 3D). The cumulative effect of the smaller WWE1 is that the binding pocket is much shallower. Indeed, the shape of WWE1 does not resemble a binding pocket compared with WWE2 (Figure 3E). Thus, the WWE1 domain lacks essentially all the WWE2 properties responsible for interaction with ADPr/ATP.

Our initial work indicated that hP13 and mP13 bind to ATP and ADPr, and we thus used these ligands to aid in crystallization efforts and to provide insights into ZnF5-WWE1-WWE2-binding properties. However, we noted that other WWE domains, such as the well-studied WWE domain from RNF-146, do not bind ADPr (Wang et al., 2012). Rather, RNF-146 is capable of binding iso-ADPr, a molecule in which the adenosine
monophosphate (AMP)-ribose is connected to a ribose phosphate, thus modeling the primary ribose-ribose (2'-1") linkage of poly(ADPr) (Figure 3F) (Wang et al., 2012). A comparison of the RNF-146 WWE/iso-ADPr structure (PDB: 3V3L) (Wang et al., 2012) and the mP13 WWE2/ADPr structure revealed important differences that are likely to explain the distinct binding properties (Figures 3F and 3G).

AMP occupies similar positions in both structures, with the adenine base sandwiched between hydrophobic residues W585, Y633, and A628 in WWE2 and Y107, Y144 and I139 in RNF-146 (Figure 3F). A major difference is a 16-residue loop in WWE2, composed of residues Q594 to N608 (Figure 3F), which is entirely missing in RNF-146. Instead, RNF-146 has a C-terminal loop that forms part of the binding site for the ribose phosphate group. The 16-residue loop of WWE2 extends into the binding cavity to contribute residues that directly engage the adenosine ribose, and in this position the loop sterically prohibits the possibility of a second ribose group (Figures 3F and 3G). The 16-residue loop therefore narrows the opening of the ligand binding cavity of mP13 WWE2 relative to the WWE of RNF-146, which has a widened WWE opening to accept a second ribose group (Figure 3G). Hence RNF-146 appears to be dependent on the second ribose phosphate to establish efficient binding. In contrast, WWE2 of mP13 appears tailored to recognize a terminal adenosine ribose without a ribose extension, and in fact would appear to disfavor the ribose-ribose linkage, without some form of adaptation in the positioning of the 16-residue loop. The 16-residue loop was not completely modeled in the hP13 ZnF5-WWE1-WWE2 crystal structure (Figure 2A) (Xue et al., 2022), presumably because of disorder, suggesting that this region has a certain level of mobility.

**Binding properties of ZnF5-WWE1-WWE2**

We first measured the binding capacity of hP13 ZnF5-WWE1-WWE2 with a fluorescence polarization (FP) assay using ATP labeled with fluorescein on the γ-phosphate (ATP-FAM), because the γ-phosphate was largely free of contacts in the ZnF5-WWE1-WWE2/ATP structure (no protein atoms within 4 Å ). ATP-FAM is a commercially available reagent that provided a reasonable ADPr analog based on our structures. hP13 ZnF5-WWE1-WWE2 interacted with the ATP-FAM with an apparent binding affinity of 97.1 ± 10.4 nM (Figure 4A). In contrast, RNF-146 showed no evidence of binding to ATP-FAM in our FP assay (Figure 4A), consistent with a published report (Wang et al., 2012). We also tested whether we could abolish the binding activity of WWE2 through mutagenesis, in which we targeted the central W611 in the binding cavity. The mutant W611A of hP13 ZnF5-WWE1-WWE2 showed no evidence of binding to ATP-FAM, consistent with WWE2 representing the primary binding site (Figure 4A). We also assessed binding using a differential scanning fluorimetry (DSF) assay to monitor relative thermal stability in the absence/presence of ligand, with the expectation that interaction with ligand would increase thermal stability (Figure 4B). We indeed observed an increase in the thermal stability of hP13 ZnF5-WWE1-WWE2 in the presence of ATP and ADPr. In contrast, the W611A mutant did not show an increase in thermal stability in the presence of ATP/ADPr. The W611A mutant exhibited a decrease in thermal stability relative to wild type, suggesting that the mutation in the central cavity has somewhat altered protein stability. However, we observed no problems in overexpressing and purifying this mutant, suggesting that the overall fold is intact.
Collectively, the data support the conclusion that the WWE2 domain serves as the primary interaction site with ADPr and ATP, and that ZnF5 and WWE1 do not contribute to this binding activity.

We also used DSF to evaluate hP13 ZnF5-WWE1-WWE2 interaction with ADPr compared with iso-ADPr. Based on structural comparison (Figures 3F and 3G), iso-ADPr was predicted to be a poor fit to the binding pocket of WWE2, because of a 16-residue loop blocking the extension of the second ribose of iso-ADPr. Over a series of concentrations, we observed again that ADPr increased hP13 ZnF5-WWE1-WWE2 thermal stability (Figure 4C). We also observed an increase in thermal stability in the presence of iso-ADPr, but to a lesser extent than seen with ADPr, and the change in thermal stability required higher concentrations of iso-ADPr. The DSF results suggest a more robust interaction with ADPr, but that iso-ADPr is nonetheless capable of forming an interaction. We have not been able to co-crystallize ZnF5-WWE1-WWE2 with iso-ADPr, which could reflect that the interaction is unfavorable. We infer that the 16-residue loop is capable to some extent of adapting to the structure of iso-ADPr, but that the WWE2 binding cavity favors ADPr.

**PAR-binding properties of ZnF5-WWE1-WWE2**

We first analyzed PAR binding through an FP-based competition assay. Based on the structural observation that the WWE2 binding pocket appeared best suited for engaging a single ADPr unit, we were particularly interested in ZnF5-WWE1-WWE2 interaction with PAR of different lengths. The relative affinity of different ADPr ligands was assessed by their capacity to outcompete ATP-FAM binding to hP13 ZnF5-WWE1-WWE2, with the expectation that a competitor would lower the FP signal. The concentration-dependent change in FP signal was modeled with a three-state competitive binding model to estimate binding affinities ($K_D$) for the competitor ligands (Figure S4A).

Due to the chemical release of PAR from protein, purification protocols primarily result in PAR chains that start with AMP linked to subsequent ADPr units. Thus, the PAR chains are of the form AMP-(ADPr)$_n$, where $n$ represents the number of complete ADPr units. The ligand AMP-(ADPr)$_2$ has two ribose-ribose linkages representative of PAR, one connecting AMP to the first ADPr and the second attaching the two ADPr units (Figure 5A). In the competition experiment, ADPr alone and PAR chains AMP-(ADPr)$_{1-9}$ were used as competitor ligands. These experiments indicated a length dependence for ZnF5-WWE1-WWE2 interaction with PAR. ADPr and AMP-(ADPr)$_1$ had similar apparent $K_D$ values of 971 and 1,179 nM, whereas AMP-(ADPr)$_2$ exhibited a ~5-fold increase in affinity with a $K_D$ of 218 nM. As the length increased, the $K_D$ values were further lowered, with AMP-(ADPr)$_9$ having an apparent $K_D$ of 3.8 nM. However, the relative changes in affinity largely stabilized with the ligand AMP-(ADPr)$_4$ and beyond, suggesting a minimal footprint for efficient binding.

We also directly measured the PAR-binding capacity of hP13 ZnF5-WWE1-WWE2 using fluorescently labeled versions of an AMP-(ADPr)$_{18}$ PAR chain in FP-binding assays. The enzymatic labeling of terminal ADP-ribose (ELTA) method labels the terminal ADPr of a PAR chain with dATP (Ando et al., 2019); thus, we added a dAMP-fluorescein group to the 2′ hydroxyl (Figure 5A) of purified AMP-(ADPr)$_{18}$ (referred to as PAR 2′ FAM). However,
given our model of WWE2 making direct contacts with the terminal adenosine group of a PAR chain, we also created a PAR molecule labeled on the opposite 1″ end of the chain (Figure 5A) with fluorescein (referred to as PAR 1″ FAM). Labeling of the 1″ end takes advantage of the AMP group that remains after PAR is released from proteins using base treatment (Abraham et al., 2020).

hP13 ZnF5-WWE1-WWE2 was estimated to bind PAR 1″ FAM with a $K_D$ of $68 \pm 34 \text{ nM}$ and to bind PAR 2′ FAM with a $K_D$ of $278 \pm 64 \text{ nM}$ (Figures 5C, 5D, S4B, and S4C), thus exhibiting a 4-fold difference in affinity. We interpret this difference in affinity to reflect that the dAMP-fluorescein from ELTA labeling on the 2′ end has created a conflict with the 16-residue loop of WWE2 that forms direct contacts with the adenosine ribose (Figures 3C and 3D). Notably, RNF-146 bound to both PAR chains with similar $K_D$ values of $4.9 \pm 0.97$ and $3.98 \pm 0.72 \text{ nM}$ (Figures 5C and 5D), consistent with the observation that RNF-146 does not specifically engage the terminal adenosine ribose. The W611A mutant of hP13 ZnF5-WWE1-WWE2 showed no evidence of stable interaction with either of the labeled PAR molecules (Figures 5C and 5D), suggesting that WWE2 represents the dominant site of interaction with PAR.

The $K_D$ of ~68 nM measured for PAR 1″ FAM is notably higher than the apparent $K_D$ of ~4 nM that was determined for the longest PAR tested in competition experiments, AMP-(ADPr)$_9$ (Figure 5B). We expect that this difference is at least in part due to the assumption in the binding model that ATP-FAM and PAR binding sites are the same, whereas in reality the PAR binding site is expected to be more expansive.

**Structural and mutagenic evidence for an extended PAR binding site**

The PAR-binding experiments indicated that ZnF5-WWE1-WWE2 interaction was enhanced by longer PAR chains (Figure 5B). However, if ZnF5-WWE1-WWE2 was solely engaging the repeating features of a PAR chain, for example, the ribose-ribose linkage as for RNF-146, then the binding should be insensitive to modifications to the PAR ends, which was not the case for ZnF5-WWE1-WWE2 but was the case for RNF-146 (Figures 5C and 5D). We therefore considered that ZnF5-WWE1-WWE2 is likely engaging the terminal end of the PAR chain, as well as an extended portion of the polymer. Inspection of the shape and electrostatic properties of mP13/hP13 ZnF5-WWE1-WWE2 suggested a surface groove that could function as an extended PAR binding site (Figure 6A). Extending from the ADPr/ATP binding cavity of WWE2, a positively charged groove is traced along the surface of WWE2 and continues to a deepened region of the groove at the junction of WWE2 and WWE1. The deepened region of the surface groove exhibits a high density of positive surface charge, and we anticipate that this site represents a major point of contact with PAR chains that extend from an anchor point in WWE2 (Figure 6A). It is compelling that the distance between the WWE2 binding site and the deep region of the surface groove appears to set a minimal length requirement for PAR chains to engage both sites, consistent with our evaluation of PAR competitor ligands. Sequence analysis of the ZnF5-WWE1-WWE2 region from multiple species indicates strong conservation within the groove along the outside of the WWE1 fold, whereas the non-functional binding cavity of WWE1 exhibited low sequence conservation (Figures S3A and S3B). We can thus speculate that the function
of the WWE1 fold is to contribute surface residues to form the extended groove, rather than contributing a functional binding cavity as seen in WWE2. We also note that in each of the mP13 crystal structures determined, a phosphate ion was modeled at the edge of the deep groove, suggesting a possible binding site for additional ADPr units (Figure 6A, inset). In particular, conserved residue R656 (R682 in hP13) engages the phosphate ion. Taken together, we propose that ZnF5-WWE1-WWE2 anchors preferentially on the terminal end of a PAR chain using the WWE2 binding cavity, and PAR chains of at least four or five units will have the capacity to form a more stable interaction by engaging the surface groove (Figure 6A).

To interrogate the extended PAR binding site, we created structure-based mutants in hP13 ZnF5-WWE1-WWE2 that were designed to specifically influence the extended PAR binding site rather than the binding pocket in WWE2, which was already tested. We created individual alanine mutants of two arginine residues, R577 and R682, and then compared their binding affinities for ATP-FAM and for PAR $^1$FAM to those of wild-type hP13 ZnF5-WWE1-WWE2. Our expectation was that these Arg residues near the groove would have a greater impact on PAR binding than on ATP-FAM binding, because they are located away from the WWE2 binding pocket. R682A and R577A exhibited ATP-FAM binding affinities similar to that of wild type (1.2- and 1.1-fold weaker; Figure 6B). In comparison, the binding affinities of R682A and R577A for PAR $^1$FAM were 4.6- and 3.2-fold weaker than wild type (Figure 6C), indicating that these perturbations to the surface groove have a more pronounced effect on PAR binding, as predicted in our model for PAR interaction.

**DISCUSSION**

Our crystal structure of mP13 ZnF5-WWE1-WWE2 indicated a dimer assembly of these domains; however, biophysical analysis indicated that the compact, monomeric arrangement of mP13 ZnF5-WWE1-WWE2 is the predominant form in solution. This conclusion is consistent with the reported crystal structure of hP13 ZnF5-WWE1-WWE2 and the AlphaFold model for this region of mP13. SEC-MALS analysis indicated a small population of ZnF5-WWE1-WWE2 dimers, and we cannot rule out the possibility that the dimer of ZnF5-WWE1-WWE2 observed in the crystal structure could represent a conformation adopted under certain conditions, for example, when PARP13/ZAP acts to signal the detection of viral RNA. In the crystallographic mP13 dimer, the WWE domains form the same interfaces seen in the compact monomer conformation, but they originate from different polypeptides. The major new contacts in the dimer state involve the linker region of both molecules. The linker regions form an extended interface in the dimer, and in the compact monomer the linker folds back onto itself. It is possible that flexibility of the linker could allow the WWE domains to exist in alternate conformations, but further studies are warranted to establish the relevance of the dimer state and the potential connection to PARP13 cellular function.

Our co-crystal structures with ATP and ADPr have provided the first views of ligands bound to ZnF5-WWE1-WWE2. The central cavity of WWE2 and surrounding loops form the binding site that primarily engages the adenosine group of ATP and ADPr. The WWE2
binding site is distinct from the WWE domain of RNF-146, which is tailored to recognize
the ribose-ribose linkage of poly(ADPr) (Wang et al., 2012). Rather, WWE2 engages the
ribose group of adenosines in a manner that should disfavor a ribose extension. WWE1
lacks entirely the central binding cavity, and the structure appears to contribute structurally
to the extended PAR binding site that we have inferred through binding analysis and that is
supported by structural analysis and mutagenesis (Figures 6A–6C).

Collectively, our analysis supports a PAR binding site that extends beyond the WWE2
cavity observed in the crystal structure. The extended PAR binding site was evident in
the competition assay in which PAR molecules required a certain length before exhibiting
efficient competition. Labeling of PAR on the terminal ADPr group using the ELTA method
introduces a fluorescein-dAMP group that would interfere with the narrow pocket of the
WWE2 cavity. Indeed, PAR labeled in this manner exhibited 4-fold weaker binding affinity
than PAR labeled on the other end (70 versus 280 nM). For PAR 2′FAM bearing the
obstruction on the terminal ADPr, we attribute the 280 nM binding affinity to at least
partially arise from the extended binding groove on ZnF5-WWE1-WWE2, although the
protein could still anchor on the ELTA end, albeit with less efficiency.

ZnF5 is suggested to have a structural role that supports the overall assembly of ZnF5-
WWE1-WWE2, and it is likely not an RNA binding element like the N-terminal Zn
fingers of PARP13. Indeed, using FP RNA-binding experiments, we determined that the
ZnF5-WWE1-WWE2 fragment is unable to bind to the RNA that is bound by the N-terminal
Zn fingers (Figure S4D) (Luo et al., 2020).

RNF-146 recognition of PAR was not influenced by differences in the label location on
PAR (Figures 5C and 5D), because the ribose-ribose linkage is the PAR feature recognized
with its WWE domain, rather than the terminal adenosine group. ZnF5-WWE1-WWE2 thus
represents a new mode of WWE engagement of PAR that is sensitive to the structure of the
terminal end of the chain and to the length of the chain. These features could tune PARP13
cellular function to be responsive to modifications of PAR structure, including modification
of the terminal end by enzymes such as OAS1, or changes in chain length through the action
of PAR-degrading enzymes. The dependency on the length of the PAR chain as a means to
both enhance binding affinity and extend the binding surface farther than the ADPr binding
pocket has been noted in studies performed on the macrodomain of ALC1 (Singh et al.,
2017).

In summary, our study provides new structural and functional insights into the PAR-binding
properties of PARP13/ZAP. The structure and binding studies provide new avenues for
investigating the cellular functions of PARP13/ZAP, including the contribution of PAR
binding and potentially aspects regulating conformational changes in response to the
detection of viral RNA.

**Limitations of the study**

The presented binding studies and mutagenesis support our model for PARP13/ZAP
engagement of PAR; however, a structure of ZnF5-WWE1-WWE2 bound to PAR is needed
to fully understand the mode of interaction. The crystallized dimer of mP13 ZnF5-WWE1-
WWE2 could potentially represent a meaningful conformation, but further investigation is required to address this possibility.

**STAR★METHODS**

**RESOURCE AVAILABILITY**

**Lead contact**—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, John M. Pascal (john.pascal@umontreal.ca).

**Materials availability**—All unique/stable reagents generated in this study are available from the lead contact with a completed materials transfer agreement.

**Data and code availability**

- The atomic coordinates and structure factors have been deposited in the Protein Data Bank (https://www.rcsb.org) with the following accession codes: PDB: 7SZ2, mP13 ZnF5-WWE1-WWE2 with ATP and PDB: 7SZ3, mP13 ZnF5-WWE1-WWE2 with ADPr and are publicly available as of the date of the publication (see the key resources table).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Bacterial and cell strains**—The product of QuickChange mutagenesis were transformed into DH5-alpha electrocompetent E. coli cells (Goldbio). Rosetta 2 (DE3) strain of E. coli (Milipore) cells were transformed with expression plasmids containing the gene of interest for protein overexpression and purification.

**METHOD DETAILS**

**Gene cloning and mutagenesis**—hp13 ZnF5-WWE1-WWE2 (residues 507 to 699) was cloned as a His-tagged, GB1 (B1 domain of Streptococcal protein G) fusion protein with a TEV protease cleavage site in a modified pET28 vector. A codon-optimized gene for mP13 ZnF5-WWE1-WWE2 (residues 476 to 673) was cloned as a His-tagged, SMT (SUMO-like tag) fusion protein in a pET28a vector (Genscript). The four N-terminal zinc fingers of hp13 (NZAP; residues 1 to 225) were cloned with a His-tag in the pET28 vector. The Quick-Change protocol was used to perform mutagenesis, which was confirmed by automated Sanger DNA sequencing. The third zinc finger of PARP1 was produced from a pET28 vector (Langéli et al., 2008). The WWE domain of RNF-146 was produced from a GST fusion vector (DaRosa et al., 2015).

**Protein expression and purification**—mP13 ZnF5-WWE1-WWE2 (wild-type and mutants), hp13 ZnF5-WWE1-WWE2 (wild-type and mutants), GST-RNF-146, PARP1 third zinc finger, and NZAP were expressed in Rosetta2 E. coli cells using LB medium and
IPTG induction (0.2 mM) at 16°C for 18 h. Seleno-methionine mP13 ZnF5-WWE1-WWE2 was expressed in defined media as described (Van Duyne et al., 1993). Pelleted cells were resuspended in the following buffer: 25 mM HEPES pH 8.0, 500 mM NaCl, and 0.1 mM TCEP. mP13 ZnF5-WWE1-WWE2 (wild-type and seleno-methionine) and hP13 ZnF5-WWE1-WWE2 (wild-type and mutants) were purified using Ni²⁺ affinity chromatography on a 5 mL His-trap column, gel filtration chromatography on either a Sephacryl 75 or Sepharose 200 column, and ion exchange chromatography using a 5 mL Q-Sepharose column (Cytiva). After the Ni²⁺ column, proteins were dialysed overnight at 4°C to remove 400 mM imidazole that was used for elution (25 mM HEPES pH 8.0, 500 mM NaCl, 0.1 mM TCEP). The SMT or GB1 tags were cleaved by addition of ULP1 or TEV protease, respectively, during dialysis. The cleaved and dialysed proteins were passed over a 5 mL His trap column to remove the his-tagged SMT, GB1, and proteases. The flow-through was concentrated by centrifugation and loaded for gel filtration chromatography (Superdex75 for hP13 ZnF5-WWE1-WWE2, and Sephacryl 200 for mP13 ZnF5-WWE1-WWE2). Gel filtration fractions containing proteins of interest were passed over a Q-Sepharose column, in which the protein of interest flowed through, and remaining contaminants bound to the column. The flow through of the Q-Sepharose column was concentrated by centrifugation. GST-RNF-146 was purified using glutathione-sepharose and heparin affinity, and NZAP was purified using Ni²⁺ and heparin affinity. The third zinc finger of PARP1 was purified using Ni²⁺ and heparin affinity and size exclusion chromatography (Langelier et al., 2008). Small-volume aliquots of concentrated protein were flash-frozen in liquid nitrogen and stored at −80°C.

**Crystallization and X-ray structure determination**—mP13 ZnF5-WWE1-WWE2 (0.4 mM) was incubated with 1 mM ATP or with 1 mM ADPribose (ADPr) prior to setting up crystallization plates at room temperature. Crystals appeared in 1.4 M Na/K phosphate pH 5.5 within two to three days with either ATP or ADPr. Crystals were cryo-protected by supplementing growth conditions with 30% glycerol or 30% ethylene glycol prior to flash-cooling in liquid nitrogen for diffraction experiments. X-ray diffraction data for mP13 ZnF5-WWE1-WWE2 crystals with ADPr and with ATP were collected at the CMCFBM (08B1–1) beamline at the Canadian Light Source and the data were processed using XDS (Kabsch, 2010). Seleno-methionine containing crystals of mP13 ZnF5-WWE1-WWE2 were obtained in the same conditions as above. Single-wavelength anomalous dispersion (SAD) X-ray diffraction data from a single crystal grown with ATP was collected at the Advance Light Source beamline 8.3.1 and processed using XDS (Kabsch, 2010). PHENIX AUTOSOL and AUTOBUILD were used to determine experimental phases and to partially build the structure (Adams et al., 2010). The seleno-methionine structure was then used as the starting model for the wild-type ZnF5-WWE1-WWE2 complexes with ATP and with ADPr. Manual model building was performed using COOT (Emsley et al., 2010) and model refinement was carried out using PHENIX (Adams et al., 2010).

**SEC-MALS**—Size-exclusion chromatography (SEC) was performed on an ÄKTAmicro liquid chromatography system (Cytiva). Samples at 40 μM were injected onto a pre-equilibrated Superdex 200 Increase 10/300 GL column operated at a flow rate of 0.35 mL/min with the following buffer: 25 mM HEPES pH 8.0, 150 mM NaCl, 0.1 mM...
DTT, and 1 mM EDTA. Samples then flowed in-line through a multi-angle light scattering (MALS) detector (DAWN HELEOS II, Wyatt Technology) followed by a refractive index detector (OptiLab T-rEX, Wyatt Technology). Data were analyzed with ASTRA 6.1.6.5 software (Wyatt Technology) to determine the molecular weight and the percent mass fractions of the eluting peaks. BSA was used as a standard for data collection and analysis prior to injecting the samples of interest.

**SEC-SAXS**—For SEC-SAXS, an ÅKTAmicro FPLC (Cytiva) was connected to a BioXolver SAXS system (Xenocs) equipped with a MetalJet D2+ 70 kV X-ray source (Excillum) and a PILATUS3 R 300K detector (Dectris). The samples were injected at 100 mg/mL for mP13 ZnF5-WWE1-WWE2 and at 17 mg/mL for hP13 ZnF5-WWE1-WWE2 onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (25 mM HEPES pH 8.0, 150 mM NaCl, 0.1 mM DTT, and 1 mM EDTA) operated at 0.1 mL/min. X-ray scattering data were collected in 30-s exposures (~400 total exposures) over the course of the elution profile. Average scattering of a buffer only region was subtracted from the average scattering over a protein peak to yield the buffer subtracted scattering profile. The radius of gyration ($R_g$), forward scattering intensity ($I_0$), and maximum dimension of ($D_{MAX}$) were derived using RAW software version 2.1.0 (Hopkins et al., 2017). $R_g$ values were determined using Guinier analysis, $D_{MAX}$ values were estimated using GNOM (Svergun, 1992), and molecular weights were calculated using the VC method (Rambo and Tainer, 2013), as implemented in RAW (Hopkins et al., 2017). FoxS and CRYSOL (ATSAS software version 2.8.4) were used to perform comparisons of experimental SAXS profiles to atomic models (Schneidman-Duhovny et al., 2013; Svergun et al., 1995). RAW was also used to perform DAMMIF/N and DENSS reconstructions for the SAXS profile of both mouse and human ZnF5-WWE1-WWE2 (Franke and Svergun, 2009; Grant, 2018).

**Zinc content analysis**—Protein samples at 10 μM were dialysed overnight at 4°C against 20 mM Hepes pH 8.0, 150 mM NaCl, 0.1 mM TCEP, and 1 mM EDTA. A Perkin Elmer NexION 300x was used for the quantification of zinc. ICP-MS standards certified traceable to NIST were used. All standards and blanks were prepared using ultrapure water (Milli-Q, 18.2 MΩ cm; total organic carbon <1 μg L−1) and ultra-trace nitric acid (Plasma Pure Plus, SCP Science). EPA 200.7 standard 6 purchased from High-Purity Standards was used for calibration. ICP-MS standards QCP-QCS-3 (Inorganic Ventures) and QCS-27 (High Purity Standards) were used for quality control. Yttrium (Inorganic Ventures) was used as internal standard. The isotope $^{66}$Zn was monitored. ICP-MS analyses were performed in triplicate measurements with 20 readings each and an integration time of 1 s.

**Differential scanning fluorimetry (DSF)**—DSF experiments were performed by adding 8 μM protein to a solution containing 25 mM HEPES pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1 mM TCEP, and 1xSYPRO-orange dye (Sigma-Aldrich), plus or minus the specified ligand concentrations. Iso-ADPr was a kind gift from Dr. Wenqing Xu (University of Washington). Fluorescence emission of the samples was measured on a Roche LightCycler 480 RT-PCR, as the temperature was increased from 20 to 85°C. The $T_M$ values were determined by a Boltzmann distribution fit to the data. The reported $\Delta T_M$ values represent the $T_M$ value with ligand minus the $T_M$ value without ligand.
**PAR synthesis and labeling**—PAR molecules of defined lengths were produced enzymatically and purified using boronate affinity followed by ion exchange chromatography for labeled PAR molecules (Ando et al., 2019; Tan et al., 2012), or followed by purification on 4 M urea/15% PAGE for unlabeled PAR [AMP-(ADPr)_{1-9}]. Unlabeled PAR was eluted from excised individual gel bands into 25 mM ammonium acetate pH 9, bound to a Sep-Pak C18 cartridge (Waters) and washed with water, eluted with 30% acetonitrile/25 mM ammonium acetate pH 9, evaporated/lyophilized to dryness, and then resuspended in water. AMP-(ADPr)_{18} was labeled on the 2′ end with fluorescein dAMP using OAS1 and the ELTA method (Ando et al., 2019) (referred to as PAR 2′ FAM). The 1″ end was FAM-labeled on the adenosine phosphate that remains after PAR is released from proteins using base treatment (Abraham et al., 2020). First, an alkyne-PEG1-amine linker was added to the adenosine phosphate using EDC coupling. The alkyne-PAR was purified to remove excess alkyne-PEG1-amine using the Monarch Nucleic Acid Cleanup Kit (NEB) following the recommended protocol. Second, FAM-PAR was produced through Cu(I)-catalyzed click chemistry between FAM-azide and the alkyne-PAR. Finally, the FAM-PAR was purified via ion-pair reversed-phase high performance liquid chromatography on an Agilent 1260 Infinity II system using an InertSustain C18 HP 3μm column (4.6 × 250 mm, GL Sciences) with two mobile phases. Mobile phase A consisted of 100 mM triethylammonium acetate pH 7.5 and mobile phase B consisted of 100% acetonitrile. Fractions containing FAM-PAR were dried down and then resuspended in Milli-Q water to a concentration of 10 μM. AMP-(ADPr)_{18} and AMP-(ADPr)_{20} were labeled on the 1″ end (both referred to as PAR 1″ FAM; FAM-AMP-(ADPr)_{18} was used in Figure 5C, and FAM-AMP-(ADPr)_{20} was used in Figure 6C).

**Fluorescence polarization (FP) binding and competition assays**—Fluorescent probes (PAR 1″ FAM, PAR 2′ FAM, ATP-FAM, RNA-FAM, and RNA-NB-FAM) were used at 5 nM. ATP-FAM [γ-(6-Aminohexyl)-ATP-6-FAM] was purchased from Jena Bioscience. FAM-labeled RNA was purchased from IDTDNA with the following sequences:

RNA-FAM,/5′-FAM/rArArArCrArArArArArGrArGrArArArA; RNA-NB-FAM,/5′-FAM/rArArArCrArArArArArGrArArArArArArA (NB, non-binding) (Luo et al., 2020). ATP-FAM and PAR binding experiments were performed in 12 mM HEPES pH 8.0, 25 mM KCl, 50 μg/mL BSA, 4% glycerol, and either 5.5 mM 2-mercaptoethanol or 0.1 mM TCEP. RNA binding experiments were performed in 12 mM HEPES pH 8.0, 300 mM KCl, 50 μg/mL BSA, 4% glycerol, and 0.1 mM TCEP. Probe was incubated with the indicated concentrations of protein/competitor ligand for 30 min at room temperature before taking fluorescence polarization measurements on a Victor3 Vplate reader (PerkinElmer). Measurements were buffer subtracted, and the polarization value was calculated for each measurement. For binding curves, a one-to-one binding model was fit to the data with a nonlinear regression equation using Microsoft excel solver to obtain binding constant (K_D) values. The experiments were performed at least three times, and the averages and standard deviations are reported. For FP competition assays, hP13 ZnF5-WWE1-WWE2 was used at 100 nM to provide a substantial population of bound ATP-FAM (roughly 50%) and thus a stable FP measurement. ADPr and PAR ligands were included at the designated...
concentrations as competitor ligands that diminish the signal of ATP-FAM binding to hP13 ZnF5-WWE1-WWE2.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Binding affinity data from fluorescence polarization experiments using PAR 1′ FAM, PAR 2′ FAM, ATP-FAM, RNA-FAM, and RNA-NB-FAM were fitted with a quadratic 1:1 binding model using MATLAB (MathWorks). The reported binding constants (K_D) and errors are the averages of results from at least three independent experiments and their calculated standard deviations, respectively. For fitting the competition assay data, a three-state competitive binding model (Champagne et al., 2009; Wang, 1995) was fit to the concentration-dependent change in FP signal using MATLAB (MathWorks), yielding an apparent K_D value for each competitor ligand. The experiments were performed at least three times, and the averages and standard deviations are reported.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- X-ray structures and SAXS analysis of the PARP13/ZAP poly(ADP-ribose) binding region
- Zinc-finger and tandem WWE domains form a compact assembly to engage poly(ADP-ribose)
- WWE2 has a functional binding site for ADP-ribose/ATP, whereas WWE1 is non-functional
- PARP13 spans multiple ADP-ribose units with a preference to anchor on terminal units
Figure 1. Crystal structure of mP13 ZnF5-WWE1-WWE2 bound to ADPr
(A) Domain organization of human PARP13 (hP13)/ZAP. The ZnF5-WWE1-WWE2 residue
boundaries for hP13 are 507–699 and for mP13 are 476–673.
(B) Crystal dimer. The crystallographic asymmetric unit contains two molecules of mP13
ZnF5-WWE1-WWE2 (red, molecule A; green, molecule B). Both molecules have an ADPr
molecule bound to WWE2. Residues C488 and C496 form part of ZnF5, which is partially
ordered in this structure.
(C) Crystal monomer. One ZnF5-WWE1-WWE2 molecule from the asymmetric unit is shown, with the extended linker (purple), WWE domains (green), ZnF5 (deep blue), and residues leading to the catalytic domain (gray) labeled.

(D) Compact monomer. Crossing over between the linker regions of the two protein molecules (small dashed line labeled “linker”) models a compact arrangement of WWE1 and WWE2 from different chains (compact monomer in blue).

(E) Model for the compact monomer with the modified linker in purple, the WWE domains in green, ZnF5 region in deep blue, and residues leading to the CAT domain in gray.
Figure 2. Biophysical analysis of PARP13 ZnF5-WWE1-WWE2
(A) Alignment of the hP13 ZnF5-WWE1-WWE2 crystal structure (PDB: 7KZH) with the compact monomer model for mP13 (this study). hP13 is colored dark gray, except for the ZnF5 region in brown, and two regions that are dissimilar between the two structures are shown in green (one is the long loop in the WWE2 binding site, and the other is the linker connecting WWE1 and WWE2). mP13 is colored light gray, except for the partially ordered ZnF5 in red, and the dissimilar regions are in blue. The bound Zn atom is shown as a sphere. The inset highlights the differences in the ZnF5 conformation, the major difference between the two structures.
(B) SEC-MALS analysis of ZnF5-WWE1-WWE2 from hP13 (blue) and mP13 (red) at 40 μM concentration. The light scattering data are plotted as the Rayleigh ratio (left y axis) versus elution volume from gel filtration. The mass estimation (right y axis) is shown across the indicated peaks.
(C) Zn content analysis was performed on the designated samples, and the relative concentrations between samples are shown for two measurements each. Proteins were analyzed at 10 μM. A Zn concentration of 10 μM was set as 100, such that the values for protein samples represent the percent of molecules with bound Zn. hP13 and mP13 refer
to the ZnF5-WWE1-WWE2 fragment. RNF-146 is a control protein that does not contain a Zn finger, ZnF3 of hP1 is a control protein that does contain a Zn finger, and the dialysis buffer was used to estimate background levels of Zn.
Figure 3. Ligand-binding properties of the WWE2 domain
(A) ATP bound within the cavity of WWE2. A weighted 2F₀-Fₐ electron density map is shown around the ATP molecule and is contoured to the 1.5σ level.
(B) ADPr bound within the cavity of WWE2. A weighted 2F₀-Fₐ electron density map is shown around the ADPr molecule and is contoured to the 1σ level.
(C) A view of key WWE2 residues interacting with ADPr. Interatomic distances (Å) are indicated next to dashed lines connecting atoms that make close contacts.
(D) Structure of WWE1 (blue/gray) superimposed on WWE2/ADPr of mP13 (green/gray). The similar structural features are colored gray, whereas the unique features are color coded. 
(E) Surface representation of WWE1 (on left in blue) illustrating the lack of a binding cavity compared with WWE2 (on right in green) with a deep cleft forming the ADPr binding pocket. 
(F) Structure of the WWE domain of RNF-146 bound to iso-ADPr (PDB: 3V3L) in pink/gray superimposed on WWE2 of mP13 bound to ADPr (green/gray). 
(G) Surface representation of WWE from RNF-146 bound to iso-ADPr (pink) superimposed on WWE2 of mP13 bound to ADPr (green).
Figure 4. Biophysical and biochemical analysis of mP13 and hP13 ZnF5-WWE1-WWE2

(A) FP binding assay using ATP-FAM and the indicated proteins. A 1:1 binding model was fit to the data (solid line, hP13 wild type [WT]). These experiments were repeated three times to provide an average $K_D$ value and associated standard deviation for hP13 WT of $97.1 \pm 10.4$ nM, whereas hP13 W611A and RNF-146 showed no indication of binding to ATP-FAM. The data points represent the mean polarization values and standard deviations for the three repeats of each experiment.
(B) DSF analysis of relative thermal stability for the indicated proteins at 8 μM concentration in the absence of ligand or in the presence of ATP or ADPr, as indicated. The two repeats of the experiment are plotted.

(C) ΔT_M (ΔT_M) comparison from DSF analysis performed with hP13 ZnF5-WWE1-WWE2 at 8 μM concentration in the absence/presence of ADPr or iso-ADPr at the indicated concentrations. The individual data points are shown for the four repeats of the experiment. The bar represents the mean value, and the error bars represent standard deviations.
Figure 5. PAR-binding properties of ZnF5-WWE1-WWE2

(A) Illustration of a PAR ligand used in this study with the termini labeled. The ribose-ribose linkage of PAR is denoted by the 2′ to 1″ linkage. The 2′ end of PAR was labeled on the 2′ OH using the ELTA method, and the 1″ end was labeled on the terminal phosphate, labeled with an asterisk.

(B) Apparent $K_D$ values determined from FP competition assays using ADPr and the indicated PAR chains. The competitor ligands were tested for their capacity to outcompete ATP-FAM probe binding to hP13 ZnF5-WWE1-WWE2 (see Figure S4A). The individual $K_D$ values from experiment repetitions are shown. The bar represents the mean $K_D$, and the error bars represent standard deviations.

(C and D) Representative curves from PAR-binding analysis using PAR FAM labeled on the 1″ end (C) or using PAR FAM labeled on the 2′ end (D). A 1:1 binding model was fit to the data (solid lines). The experiments were repeated at least three times to produce the average
K_D values and the associated standard deviations shown in (C) and (D). See Figures S4B and S4C for all binding curve replicates.
Figure 6. Evidence for a PAR binding groove

(A) Electrostatic surface potential of hP13 ZnF5-WWE1-WWE2 using crystal structure PDB: 7KZH. The putative binding groove is highlighted with units of PAR, represented as green bars, tracing along the groove on the surface of WWE1 and WWE2. The terminal ADPr in the binding pocket is designated n, and the preceding ADPr units are designated n−1, n−2, and so forth, providing a rough estimate of the distance between the WWE2 binding pocket and the deepened region of the groove. The inset panel shows features of the mP13 crystal structure surrounding the bound phosphate ion, and the hP13 structure is aligned to mP13.

(B) Fluorescence polarization binding affinities measured using ATP-FAM for the indicated proteins.

(C) Fluorescence polarization binding affinities measured using fluorescent PAR FAM labeled on the 1″ end for the indicated proteins.
For (B) and (C), the individual $K_D$ values are plotted. The bar represents the mean values, which are also listed on the plot. The error bars represent standard deviations, and they are also listed on the plot.
## Table 1.

Crystallographic data and refinement statistics

| Data collection$^d$ | mP13 ZnF5-WWE1-WWE2: ATP | mP13 ZnF5-WWE1-WWE2: ADPr |
|---------------------|---------------------------|-----------------------------|
| Structure           | PDB: 7SZ2                 | PDB: 7SZ3                   |
| PDB ID              | P3$_1$21                  | P3$_1$21                    |
| Space group         |                           |                             |
| Unit cell dimensions| $a = b = 87.5$ Å, $c = 129.8$ Å | $a = b = 87.4$ Å, $c = 128.7$ Å |
|                     | $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$ | $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$ |
| Wavelength (Å)      | 1.18                      | 1.18                        |
| Resolution range (Å)| 43.77 to 2.20 (2.27 to 2.20) | 49.04 to 2.20 (2.27 to 2.20) |
| Completeness (%)    | 100.0 (100.0)             | 100.0 (99.9)                |
| Unique observations | 29,855 (2,545)            | 29,530 (2,519)              |
| Average redundancy  | 21.8 (22.5)               | 21.9 (22.4)                 |
| Mean (I/σI)$^b$     | 33.8 (2.3)                | 28.7 (2.0)                  |
| $R_{merge}$ (%)$^b$ | 6.2 (163.9)               | 8.5 (227.0)                 |
| $R_{pim}$ (%)$^b$   | 1.4 (35.2)                | 1.8 (48.3)                  |
| Mean I CC(1/2)$^b$  | 1.0 (0.828)               | 1.0 (0.849)                 |

| Model refinement$^d$ |                   |                             |
|---------------------|-------------------|-----------------------------|
| Resolution range (Å)| 20 to 2.20 (2.25 to 2.20) | 20 to 2.20 (2.25 to 2.20) |
| No. of reflections  | 27,170 (1,958)    | 26,802 (1,915)              |
| $R^c$               | 0.2027 (0.319)    | 0.1926 (0.380)              |
| $R_{free}^c$        | 0.2384 (0.340)    | 0.2305 (0.367)              |
| No. of atoms/average B factor (Å$^2$) | 3,236/66.0 | 3,230/63.0 |
| Protein             | 3.003/65.9        | 2.998/62.6                  |
| Solvent             | 171/75.5          | 160/72.20                   |
| Ligand              | 62/81.2           | 72/77.7                     |
| Phi/Psi, preferred (%)/outliers (n)$^d$ | 94.22/0         | 95.07/0                     |
| RMSD bond angles (°) | 1.4375 | 1.5275 |
|---------------------|--------|--------|
| RMSD bond lengths (Å) | 0.0068 | 0.0082 |

RMSD, root-mean-square deviation.

Values in parentheses refer to data in the highest resolution shell.

As calculated in SCALA (Winn et al. 2011):  \( R_{\text{merge}} = \frac{\sum_{hkl} \sum_j |I_j - \langle I \rangle|}{\sum_{hkl} \sum_j I_j} \) \( \langle I \rangle \) is the mean intensity of observations of reflection \( hkl \) and its symmetry equivalents; \( R_{\text{pim}} \) takes into account measurement redundancy when calculating \( R_{\text{merge}} \); mean I CC(1/2) is the correlation between mean intensities calculated for two randomly chosen half-sets of the data.

\( R = \frac{\sum_{hkl} |F_{\text{obs}} - k F_{\text{calc}}|}{\sum_{hkl} |F_{\text{obs}}|} \) for reflections used in refinement. \( R_{\text{free}} = R \) for 5% of reflections excluded from crystallographic refinement.

As reported in Crystallographic Object-Oriented Toolkit (COOT) (Emsley et al., 2010).
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| E. coli strain BL21 (DE3) Rosetta2 | Millipore | Cat#71400 |
| **Biological samples** | | |
| hP13 ZnF5-WWE1-WWE2 (residues 507–699) WT protein | This paper | N/A |
| mP13 ZnF5-WWE1-WWE2 (residues 476–673) WT protein | This paper | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| PARP1 3rd Zinc finger | (Langelier et al., 2008) | N/A |
| WWE of RNF146-GST tagged | (Wang et al., 2012) | N/A |
| ADPr | Sigma-Aldrich | Cat#A0752 |
| ATP | Sigma-Aldrich | Cat#A2383 |
| Iso-ADPr | Dr. Wenqing Xu, University of Washington | N/A |
| 18-mer PAR FAM labeled on 2′ end (PAR 2′ FAM) | (Ando et al., 2019) | N/A |
| 18-mer PAR FAM labeled on 1″ end (PAR 1″ FAM, AMP-(ADPr)₁₈) | (Abraham et al., 2020) | N/A |
| 20-mer PAR FAM labeled on 1″ end (PAR 1″ FAM, AMP-(ADPr)₂₀) | (Abraham et al., 2020) | N/A |
| Unlabeled PAR, AMP-(ADPr)₁₋₉ | This paper | N/A |
| ATP-FAM labeled on gamma-phosphate | Jena-Bioscience | Cat#NU-833-5FM |
| **Deposited data** | | |
| mP13 ZnF5-WWE1-WWE2 (residues 476–673): ATP | This paper | PDB: 7SZ2 |
| mP13 ZnF5-WWE1-WWE2 (residues 476–673): ADPr | This paper | PDB: 7SZ3 |

| **Oligonucleotides** | | |
| RNA-FAM with a 6-FAM group on the 5′ end | IDT | N/A |
| RNA-NB-FAM with a 6-FAM group on the 5′ end | IDT | N/A |

| **Recombinant DNA** | | |
| hP13 ZnF5-WWE1-WWE2 (residues 507–699) WT-Gb1 tag | This paper | N/A |
| hP13 ZnF5-WWE1-WWE2 (residues 507–699) W611A-Gb1 tag | This paper | N/A |
| hP13 ZnF5-WWE1-WWE2 (residues 507–699) R577A-Gb1 tag | This paper | N/A |
| hP13 ZnF5-WWE1-WWE2 (residues 507–699) R684A-Gb1 tag | This paper | N/A |
| mP13 ZnF5-WWE1-WWE2 (residues 476–673)-SMT | This paper | N/A |
| NZAP, hP13 zinc finger 1–4 (residues 1–225) | This paper | N/A |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| WWE of RNF146-GST tagged | (Wang et al., 2012) | N/A |
| Software and algorithms | | |
| Astra 6.1.6.5 | Wyatt technology | https://www.wyatt.com/products/software/astra.html |
| FOXS | (Schneidman-Duhovny et al., 2013) | https://modbase.compbio.ucsf.edu/foxs |
| CRYSOL | (Svergun et al., 1995) | https://www.embl-hamburg.de/biosaxs/manuals/crysol.html |
| BioXTAS RAW | (Hopkins et al., 2017) | https://bioxtas-raw.readthedocs.io/ |
| DENSS | (Grant, 2018) | https://www.tdgrant.com/denss/ |
| XDS | (Kabsch, 2010) | https://xds.mr.mpg.de |
| COOT | (Emsley et al., 2010) | https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/ |
| PHENIX | (Adams et al., 2010) | http://www.phenix-online.org |
| MATLAB | MathWorks | https://www.mathworks.com/products/matlab.htm |