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An Allosteric Interaction Links USP7 to Deubiquitination and Chromatin Targeting of UHRF1

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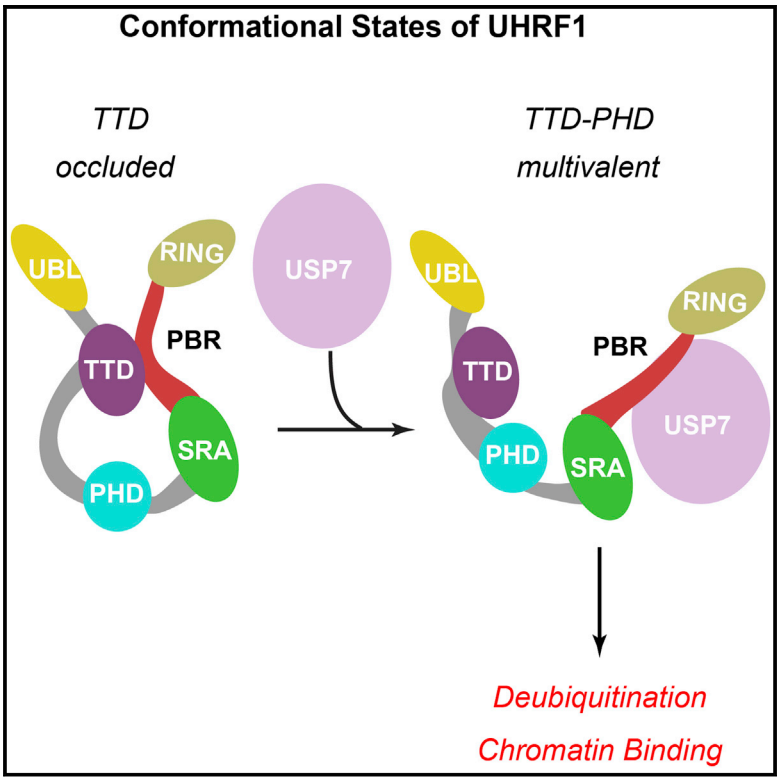
Graphical Abstract



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In Brief

Zhang et al. report the crystal structure of USP7 ubiquitin-like domains in complex with the UHRF1 polybasic region. Structural analysis, combined with biochemical and cellular analysis, reveals that the USP7 interaction influences both ubiquitination and chromatin association of UHRF1.

Highlights

- USP7 ubiquitin-like domains bind to the UHRF1 polybasic region
- USP7 interaction promotes USP7-mediated deubiquitination of UHRF1
- USP7 allosterically regulates the conformational states of UHRF1
- USP7 interaction affects the chromatin association of UHRF1

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Report

An Allosteric Interaction Links USP7 to Deubiquitination and Chromatin Targeting of UHRF1

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SUMMARY

The protein stability and chromatin functions of UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1) are regulated in a cell-cycle-dependent manner. We report a structural characterization of the complex between UHRF1 and the deubiquitinase USP7. The first two UBL domains of USP7 bind to the polybasic region (PBR) of UHRF1, and this interaction is required for the USP7-mediated deubiquitination of UHRF1. Importantly, we find that the USP7-binding site of the UHRF1 PBR overlaps with the region engaging in an intramolecular interaction with the N-terminal tandem Tudor domain (TTD). We show that the USP7-UHRF1 interaction perturbs the TTD-PBR interaction of UHRF1, thereby shifting the conformation of UHRF1 from a TTD-"occluded" state to a state open for multivalent histone binding. Consistently, introduction of a USP7-interaction-defective mutation to UHRF1 significantly reduces its chromatin association. Together, these results link USP7 interaction to the dynamic deubiquitination and chromatin association of UHRF1.

INTRODUCTION

One of the fundamental principles in epigenetic regulation involves temporal and spatial control of macromolecular machineries that govern epigenetic events. Epigenetic modifications, including DNA methylation and histone modifications, are recognized by a diverse family of effector proteins (Bogdanović and Veenstra, 2009; Musselman et al., 2012) whose functions are often subject to dynamic regulation in response to environmental cues. However, how these chromatin effector proteins are regulated remains incompletely understood.

UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1), also known as ICBP90 and NP95 in mouse, is a multidomain protein that plays critical roles in regulating various processes, such as DNMT1 (DNA methyltransferase 1)-mediated DNA methylation maintenance (Bostick et al., 2007; Sharif et al., 2007). UHRF1 contains a ubiquitin-like (UBL) domain at the N terminus, followed by a tandem Tudor domain (TTD), a plant homeodomain (PHD), a SET- and RING-associated (SRA) domain, and a RING domain (Figure 1A). Among these protein modules, the PHD and TTD recognize the N-terminal tail of histone H3 unmethylated at arginine 2 (Hu et al., 2011; Lallous et al., 2011; Rajakumara et al., 2011) and dimethylated/trimethylated at lysine 9 (H3K9me2/3) (Arita et al., 2012; Cheng et al., 2013; Karagianni et al., 2008; Rothbart et al., 2012, 2013; Xie et al., 2012), respectively, whereas the SRA domain specifically binds to hemimethylated CpG sites (Arita et al., 2008; Avvakumov et al., 2008; Bostick et al., 2007; Hashimoto et al., 2008; Sharif et al., 2007). The RING domain of UHRF1 has been shown to serve as an E3 ubiquitin ligase to promote monoubiquitination of H3 at lysine 18 and/or 23 (Nishiyama et al., 2013; Qin et al., 2015), which in turn recruits DNMT1 to replicating chromatin.

Despite these advances, the mechanism underlying the regulation of UHRF1 remains to be elucidated. Indeed, emerging evidence has shown that UHRF1 is subject to temporal and spatial control (Gelato et al., 2014; Ma et al., 2012). First, the stability of UHRF1 is regulated by ubiquitination in a cell-cycle-dependent manner (Ma et al., 2012). During S phase, deubiquitinase USP7 (ubiquitin-specific-processing protease 7) associates with UHRF1, keeping it from being ubiquitinated (Ma et al., 2012). When cells enter mitosis, USP7 dissociates from UHRF1, exposing it for ubiquitination and consequent proteasomal degradation (Ma et al., 2012). Second, the chromatin association of UHRF1 peaks in mid S phase to accompany pericentromeric heterochromatin replication (Miura et al., 2001; Papait et al., 2007; Taylor et al., 2013), which is likely mediated by a conformational transition between two alternative functional states of UHRF1 (Gelato et al., 2014). In one state, the H3K9me3-binding site of the UHRF1 TTD is occluded due to its intramolecular interaction with a C-terminal polybasic region (PBR). In the second state, the TTD is relieved from the PBR interaction and thus forms a multivalent histone-binding cassette with the PHD (Gelato et al.,

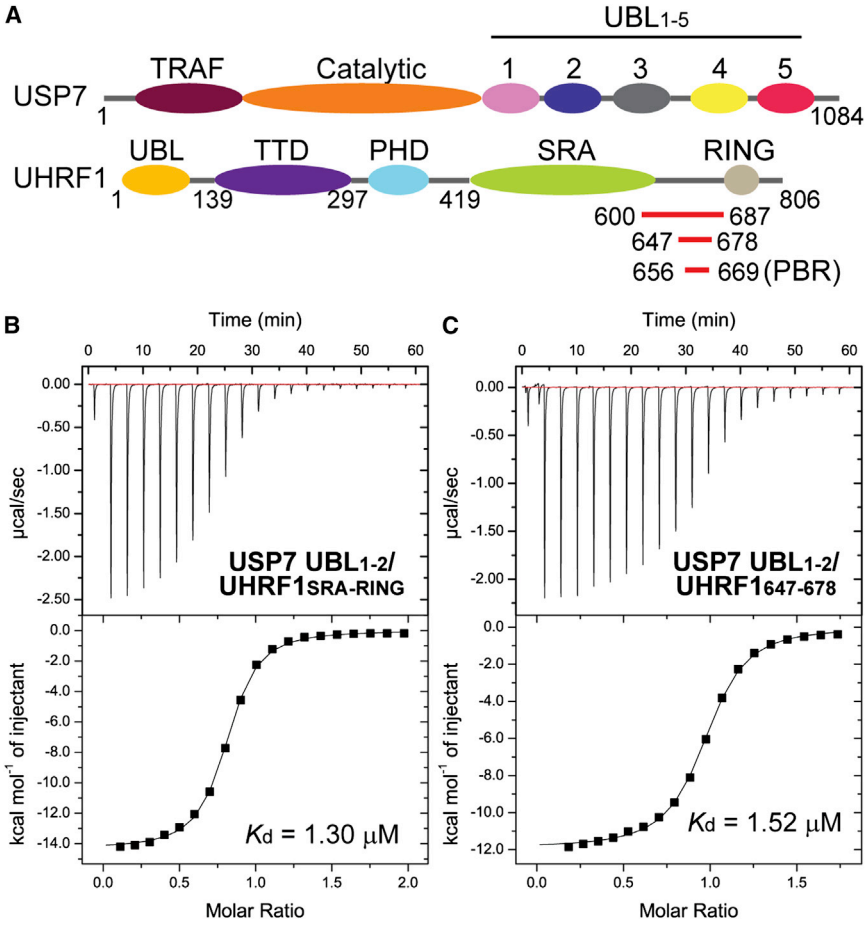

Figure 1. Identification of the UHRF1 and USP7 Interaction Domains

(A) Domain architectures of UHRF1 and USP7. The UHRF1 fragments used for biochemical analysis are labeled with residue numbers. TRAF, tumor necrosis factor receptor-associated factor.

(B and C) ITC binding curves for USP7 UBL_{1-2} over $UHRF1_{SRA-RING}$ (B) and $UHRF1_{647–678}$ (C).

See also Figure S1.

2014). Binding of the signaling molecule phosphatidylinositol 5-phosphate (PI5P) to the UHRF1 PBR switches the UHRF1 conformation from the TTD-"occluded" state to the TTD-PHD multivalent state, which leads to its increased heterochromatin association (Gelato et al., 2014). However, how networks of interactions functionally regulate UHRF1 in cells remains unresolved.

In this study, we characterized the interaction between UHRF1 and USP7 by structure determination of the UHRF1-USP7 complex. Our structural analyses reveal that the UHRF1-USP7 interaction is mediated by the first two UBL domains of USP7 and the PBR of UHRF1. Through functional assays, we show that the interaction of UHRF1 with USP7 is not only critical for its deubiquitination but also for disrupting the intramolecular TTD-PBR interaction, thereby shifting the conformation of UHRF1 to promote multivalent histone engagement of the TTD-PHD cassette. Indeed, point mutations that disrupt the USP7 interaction greatly reduce the chromatin association of UHRF1. Our study thus uncovers a novel mechanism by which USP7 promotes both the stability and chromatin association of UHRF1.

RESULTS

Characterization of the UHRF1-USP7 Interaction

A recent study suggests that the interaction between USP7 and UHRF1 is mediated by the UBL domains of USP7 and a linker region between the SRA and RING finger domains of UHRF1 (residues 600–687; $UHRF1_{600–687}$) (Figure 1A) (Ma et al., 2012). To further map the USP7 and UHRF1 interaction interface, we performed isothermal titration calorimetry (ITC) to measure the binding between truncated USP7 and UHRF1 fragments. The first two UBL domains (UBL_{1-2}) of USP7 interact directly with a fragment of UHRF1 encompassing its SRA and RING domains (residues 419–806; $UHRF1_{SRA-RING}$), with a dissociation constant (K_d) of 1.30 µM (Figure 1B). To narrow this interaction surface further, we showed that the UBL_{1-2} dual domain of USP7 binds to $UHRF1_{600–687}$ with a K_d of 1.42 µM (Figure S1A), and comparable binding activity is observed (K_d 1.52 µM) when we truncated UHRF1 further to a 32-amino acid sequence, $UHRF1_{647–678}$ (Figure 1B). By contrast, we did not observe appreciable interaction between the first UBL domain (UBL_1) of USP7 and $UHRF1_{SRA-RING}$ (Figure S1B), indicating that UBL_1 alone is insufficient for mediating the UHRF1-USP7 interaction. Collectively, these data show that the $UHRF1_{647–678}$ fragment and the USP7 UBL_{1-2} domains mediate the UHRF1-USP7 interaction.

Structure of the $UHRF1_{647–678}$-USP7 $UBL_{1–3}$ Complex

Next, to provide the molecular basis for the UHRF1-USP7 interaction, we determined the crystal structure of $UHRF1_{647–678}$ in complex with the first three UBL domains ($UBL_{1–3}$) of USP7 at 2.3-Å resolution (Table S1). It is notable that there are two UHRF1-USP7 complexes in each asymmetric unit (Figure S1C), and in both complexes the UHRF1 fragments superimpose well, except for one region (residues S652–T655) that is only observable in one of the complexes due to crystal packing (Figure S1D). The UHRF1 segment containing residues G656–R662 is anchored to an acidic surface formed by the closely packed UBL_1 and UBL_2 domains of USP7, whereas no intermolecular interaction involves the USP7 UBL_3 domain (Figures 2A and 2B). The interactions between the USP7 UBL_{1-2} domains and the $UHRF1_{647–678}$ peptide are mediated by hydrogen bonds and electrostatic attractions (Figure 2C). Of particular note, the side chain of UHRF1 K659 is inserted into an acidic pocket of USP7, formed by residues R628, E736, D764, E759, and

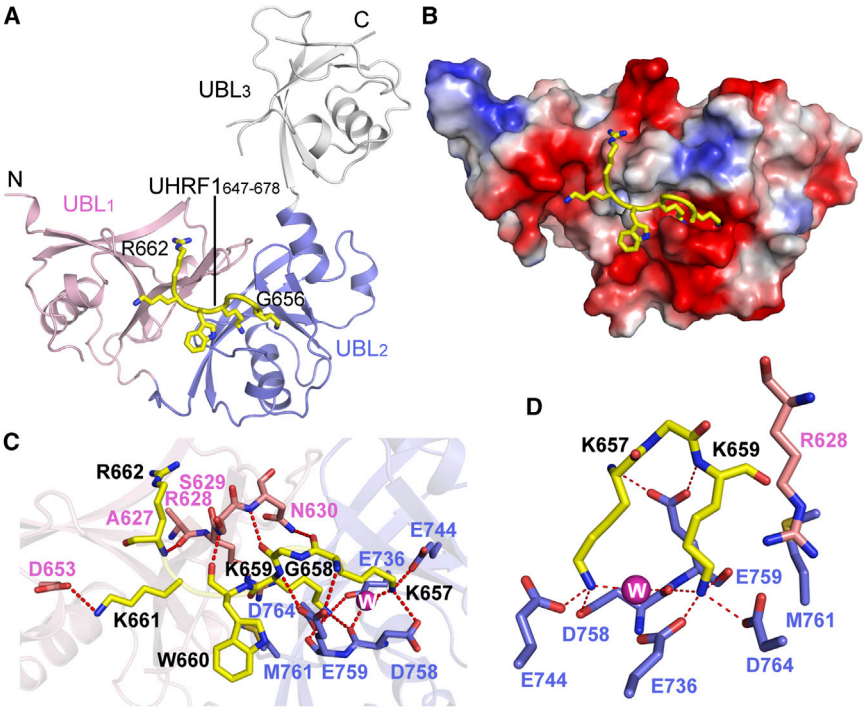

Figure 2. Structural Basis of the UHRF1$_{647–678}$-USP7 UBL$_{1–3}$ Complex

(A) Ribbon representation of the UHRF1$_{647–678}$-USP7 UBL$_{1–3}$ complex, with USP7 UBL$_1$, UBL$_2$, and UBL$_3$ colored pink, light blue, and silver, respectively. The UHRF1$_{647–678}$ fragment is shown in stick representation.

(B) Electrostatic surface representation of the UHRF1$_{647–678}$-UHRF1 UBL$_{1–3}$ complex. For clarity, the USP7 UBL$_3$ domain is not shown.

(C) Hydrogen-bonding interactions (dashed lines) between USP7 and UHRF1. The water molecule is shown as a sphere labeled "W."

(D) Close-up view of the UHRF1 K657- and K659-associated intermolecular interactions.

See also Table S1.

M761, with the K659 ε-amino group hydrogen bonded to the side-chain carboxylates of USP7 E736 and D764 and to the backbone carbonyl oxygen atoms of USP7 D758 and E759 (Figures 2C and 2D). In a parallel configuration, the ε-amino group of UHRF1 K657 forms direct hydrogen bonds with the side-chain carboxylates of USP7 E744 and D758 and a water-mediated hydrogen bond with the backbone carbonyl oxygen of USP7 D758 (Figures 2C and 2D). Meanwhile, the backbone amide groups of UHRF1 K657 and K659 each form a hydrogen bond with the side-chain carboxylate of USP7 E759 (Figures 2C and 2D). The backbone carbonyl oxygen of UHRF1 K657 forms a hydrogen bond with the side-chain amide of USP7 N630 (Figure 2C). C-terminal to UHRF1 K657 and K659, the ε-amino group of UHRF1 K661 forms a hydrogen bond with the side-chain carboxylate of USP7 D653 (Figure 2C). In addition, three main-chain hydrogen bonds, formed between UHRF1 G658, W660, and R662 and USP7 N630, S629, and A627, respectively, further support the UHRF1-USP7 interaction (Figure 2C).

Mutational Analysis of the UHRF1-USP7 Interaction

To test our structural observations, we mutated a number of key interacting residues for ITC (Table S2). In comparison with the wild-type (WT) UHRF1$_{647–687}$-USP7 UBL$_{1-2}$ interaction, UHRF1 K659E and USP7 E736A mutations reduced the binding affinity by 38- and 4-fold, respectively (Figure 3A). Furthermore, the UHRF1 K657Q/K659Q and K657E/K659E double mutations both reduced the binding affinity by over 200-fold (Figure 3A). Together, these data support our structural observations. Consistently, sequence alignment of UHRF1 protein orthologs revealed that except for K657, which is not present in NP95, the USP7-interacting residues, including UHRF1 G658, K659, and K661, are highly conserved across species (Figure 3B), suggesting an important role for these residues in UHRF1 regulation.

To test whether UHRF1 K657 and K659 are important for association of full-length UHRF1 with USP7, we ectopically expressed hemagglutinin (HA)-tagged USP7 in HEK293 cells in the presence or absence of Flag-tagged UHRF1 (WT or K657Q/K659Q mutant), followed by coimmunoprecipitation with an anti-Flag antibody (Figure 3C). We found that USP7 coprecipitates with WT UHRF1, confirming that these proteins form a complex in cells (Figure 3C). As predicted from the structural data, the UHRF1 K657Q/K659Q double mutant reduced the association of UHRF1 with USP7, further supporting a critical role for these residues in mediating the UHRF1-USP7 interaction.

Effect of the UHRF1-USP7 Interaction on USP7-Mediated Deubiquitination of UHRF1

To investigate the functional consequence of the UHRF1-USP7 interaction, we next performed in vitro ubiquitination assays, monitoring autoubiquitination of wild-type UHRF1 or the K659E mutant in the presence of full-length USP7. Consistent with previous observations (Felle et al., 2011; Ma et al., 2012), our results showed that USP7 reduced autoubiquitination activity of wild-type UHRF1 (Figure 3D). In contrast, USP7 cannot reduce the autoubiquitination of the UHRF1 K659E mutant. These data confirm previous observations that the USP7-UHRF1 interaction is required for USP7-mediated deubiquitination of UHRF1 (Felle et al., 2011; Ma et al., 2012).

Effect of the UHRF1-USP7 Interaction on the Allosteric Regulation of UHRF1

A recent study (Gelato et al., 2014) showed that an intramolecular TTD-PBR interaction of UHRF1 occludes its TTD from binding to H3K9me3. Given the fact that the USP7-binding site of UHRF1 from this study is located within the UHRF1 PBR sequence (Figure 4A) (Gelato et al., 2014), we postulated that the UHRF1-USP7 interaction might interfere with the UHRF1 TTD-PBR interaction. To test this hypothesis, we performed NMR

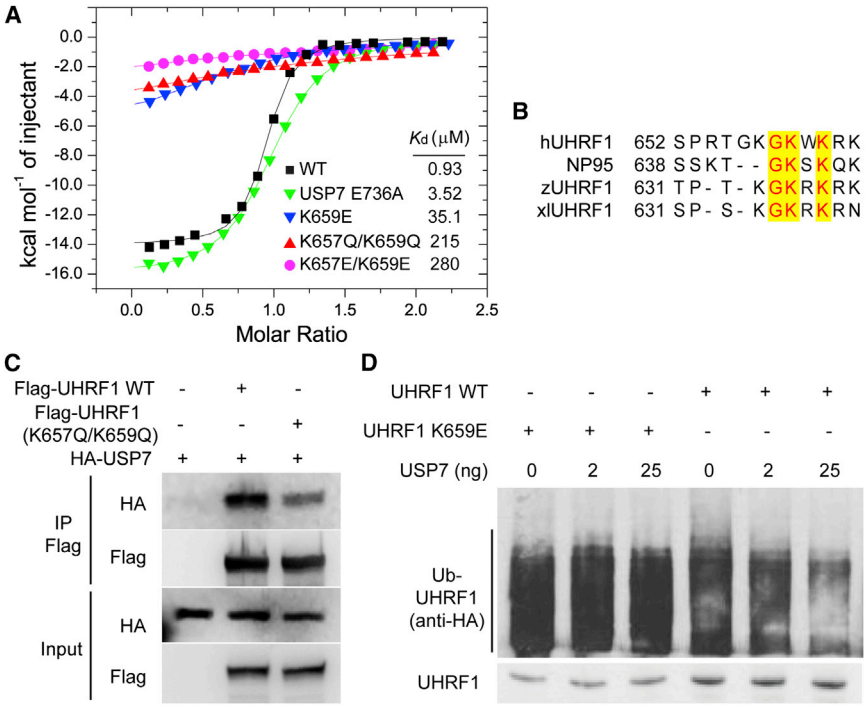

Figure 3. Biochemical Analysis of the UHRF1-USP7 Interaction

(A) ITC and mutational analysis of the $UHRF1_{647–687}$-USP7 UBL_{1-2} interaction. The mutations include USP7 E736A, UHRF1 K659E, K657Q/K659Q, and K657E/K659E.

(B) Sequence alignment of the USP7-binding motif of human UHRF1 with its mouse (NP95), zebrafish (zUHRF1), and *Xenopus laevis* (xlUHRF1) orthologs. Identical residues are colored red and highlighted in yellow.

(C) Immunoprecipitation (IP) analysis of the interaction between full-length Flag-tagged UHRF1 and HA-tagged USP7.

(D) In vitro ubiquitination assay of wild-type and K659E UHRF1 in the presence of HA-tagged ubiquitin and USP7. The ubiquitination level (upper panel) and total amount of UHRF1 (lower panel) were analyzed by anti-HA western blot and SYPRO staining, respectively.

See also Figure S4 and Table S2.

titrations to investigate whether the UHRF1 TTD-PBR interaction is affected by the USP7 UBL_{1-2} dual domain. A number of $^{1}H,^{15}N$-HSQC spectra were collected for the ^{15}N-labeled UHRF1 TTD, alone or in the presence of the UHRF1 PBR ($UHRF1_{647–687}$) and/or the USP7 UBL_{1-2} domains (Figures 4B–4D; Figure S2A). Consistent with a previous study (Gelato et al., 2014), we observed that a number of NMR peaks of the UHRF1 TTD were significantly shifted upon addition of $UHRF1_{647–687}$ with 20% molar excess (Figures 4B–4D; Figure S2A). Based on the reported chemical shift assignment of the TTD (Nady et al., 2011), we were able to assign a number of NMR peaks with large chemical shift changes to UHRF1 residues, including I224, G275, and V287. It is notable that the chemical shift perturbations of these residues by the UHRF1 PBR were also observed in a previous study (Gelato et al., 2014), and that residues I224 and V287 were conceived as part of the putative PBR-binding pocket of the TTD (Gelato et al., 2014). However, when we further mixed the NMR sample with the USP7 UBL_{1-2} dual domain (40% molar excess over the UHRF1 TTD), the majority of PBR-perturbed TTD peaks shifted back toward the peak positions corresponding to the free state (Figures 4B–4D; Figure S2A). These data suggest that the USP7 UBL_{1-2} dual domain affects the intramolecular TTD-PBR interaction of UHRF1 through direct interaction with the UHRF1 PBR. Consistently, we found that the presence of USP7 $UBL_{1–5}$ (residues 560–1084) modestly increased the binding of full-length UHRF1 to the $H3_{(1–20)}$K9me3 peptide by a pull-down assay, confirming that the USP7 interaction enhances TTD-H3K9me3 binding in the context of full-length UHRF1 (Figure S2B).

We then performed ITC for the USP7 UBL_{1-2} dual domain over a UHRF1 fragment spanning from the TTD toward the C-terminal RING domain (residues 138–806; $UHRF1_{TTD-RING}$) to further examine whether an intramolecular UHRF1 TTD-PBR interaction affects the UHRF1-USP7 interaction. Our results showed that the USP7 UBL_{1-2} dual domain binds to $UHRF1_{TTD-RING}$ with a K_d of 27.2 μM (Figure 4E), a binding affinity that is ~20-fold weaker in comparison with the binding of the USP7 UBL_{1-2} to $UHRF1_{SRA-RING}$ (Figure 4E). This result suggests that the presence of the UHRF1 TTD conversely disfavors the UHRF1-USP7 interaction.

Taken together, these findings establish that the UHRF1-USP7 interaction disrupts the intramolecular TTD-PBR interaction of UHRF1, thereby shifting the conformation of the UHRF1 TTD from the occluded state to an open state that allows multivalent histone binding by the TTD-PHD.

Effect of the USP7-UHRF1 Interaction on the Chromatin Association of UHRF1

The observation that the USP7 interaction modulates the conformational states and H3K9me3 binding of UHRF1 prompted us to investigate whether the UHRF1-USP7 interaction affects chromatin association of UHRF1. Toward this end, we tested recombinant full-length UHRF1 and a USP7-binding mutant (K657E/K659E) in histone peptide-binding assays by fluorescence polarization (Figure S3A). Consistent with our previous findings (Rothbart et al., 2013), both full-length wild-type and K657E/K659E UHRF1 were found to bind to $H3_{(1–20)}$K9me3 peptides conjugated with 5-carboxyfluorescein (5-FAM) at the C terminus similarly. By contrast, neither protein had measurable interaction with $H3_{(1–20)}$K9me3 peptides conjugated with 5-FAM at the N terminus, the peptides that were previously shown to block UHRF1 PHD-histone binding (Rothbart et al., 2013). These data, in line with a recent study (Gelato et al., 2014) that the K657E/K659E mutant alone does not affect the UHRF1 TTD-PBR interaction appreciably, suggest that the previously described multivalent histone engagement of the TTD-PHD (Rothbart et al.,

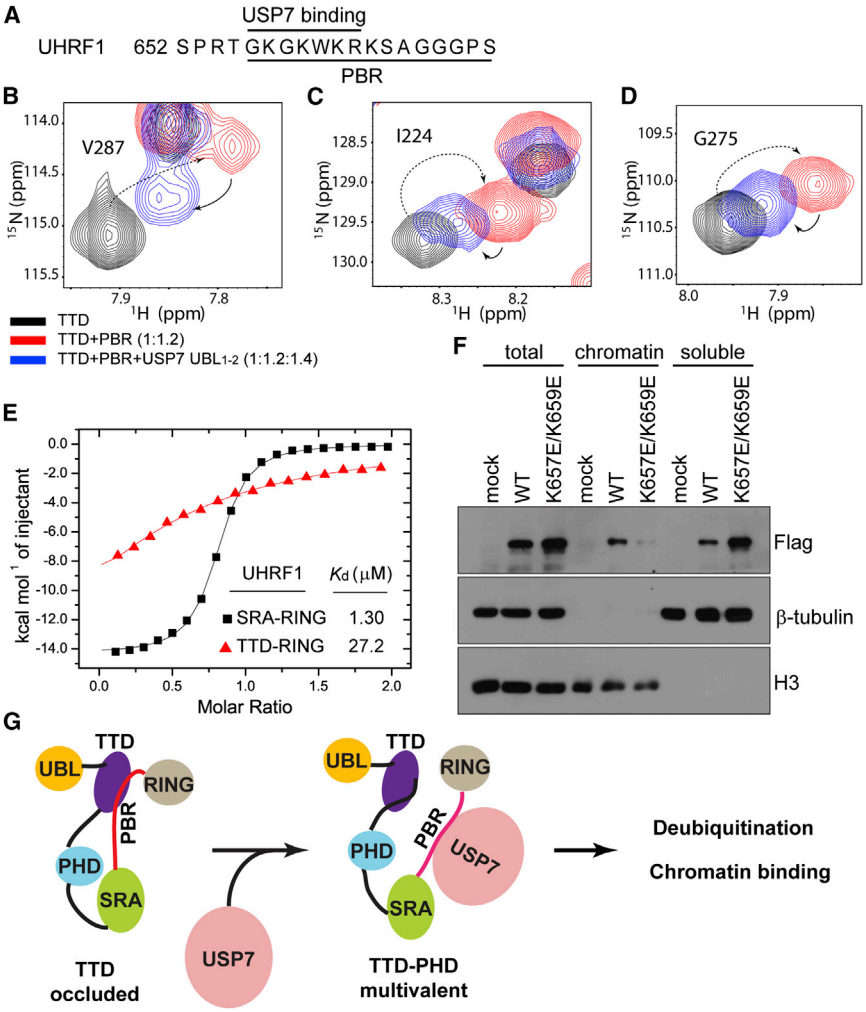

Figure 4. UHRF1-USP7 Interaction Affects UHRF1 Intramolecular Regulation and Chromatin Association

(A) Sequence of a selected UHRF1 fragment, with the USP7-binding site and PBR sequence labeled.

(B–D) Overlaid 2D $^{1}H,^{15}N$-HSQC spectra highlight the chemical shift changes of selected residues in the UHRF1 TTD. The spectra were collected for the UHRF1 TTD free (black) and in the presence of the UHRF1 PBR (red) or UHRF1 PBR and USP7 UBL_{1-2} (blue). The arrows with dashed and solid lines mark the chemical shift changes of selected residues under different conditions.

(E) ITC binding curves for USP7 UBL_{1-2} over $UHRF1_{SRA-RING}$ (black) or $UHRF1_{TTD-RING}$ (red).

(F) Chromatin association assay of Flag-tagged WT and K657E/K659E UHRF1 from asynchronously growing HeLa cells. Mock indicates no DNA control.

(G) Model for USP7 regulation. The USP7 interaction allosterically regulates the conformational states of UHRF1 and affects its ubiquitination and chromatin binding.

See also Figures S2 and S3 and Table S2.

2013) remains for full-length UHRF1 and its K657E/K659E mutant. Furthermore, we showed that unlike wild-type UHRF1, the UHRF1 K657E/K659E mutant dramatically decreases its association with chromatin isolated from HeLa cells (Figure 4F; Figure S3B). Collectively, these observations suggest that the USP7 interaction facilitates the association of UHRF1 with chromatin.

DISCUSSION

Cell-Cycle-Dependent USP7 Regulation of UHRF1

UHRF1 has emerged as an important epigenetic regulator in the maintenance of DNA methylation through regulating the recruitment and stability of DNMT1 (Bostick et al., 2007; Du et al., 2010; Sharif et al., 2007). This regulation is achieved, in part, through dynamic regulation of UHRF1—both at the level of its chromatin association and ubiquitination—throughout the cell cycle (Gelato et al., 2014; Ma et al., 2012; Taylor et al., 2013). In this study, we have confirmed the requirement of USP7 interaction for UHRF1 deubiquitination through an in vitro ubiquitination assay. More importantly, we uncovered the UHRF1 PBR, a region known to be crucial for modulating an interconversion between the two alternative functional states of UHRF1 (Gelato et al., 2014), as the region responsible for mediating its interaction with USP7. The interaction between USP7 and the UHRF1 PBR therefore may help release the TTD from the PBR-bound state, entering into a state that allows TTD-PHD multivalent histone engagement, as characterized previously (Rothbart et al., 2013). Indeed, with a series of structural, biochemical, and mutagenesis assays, we demonstrated that the USP7 interaction with the UHRF1 PBR sequence promotes H3K9me3 binding by UHRF1 through interfering with its intramolecular TTD-PBR interaction; consistently, disruption of the UHRF1-USP7 interactions leads to a significantly decreased chromatin association of UHRF1 in vivo. Taken together, this study reveals that the USP7 interaction plays a dual regulatory role in both deubiquitination and chromatin association of UHRF1 (Figure 4G).

Effects of Posttranslational Modifications on UHRF1-USP7 Association

A previous study suggested that phosphorylation of UHRF1 S652 regulates the dissociation of the UHRF1-USP7 complex in M phase (Ma et al., 2012). Interestingly, our study indicates that this residue is indeed adjacent to the USP7 interaction site. However, we did not observe any direct interaction between UHRF1 S652 and USP7. Moreover, our ITC analysis revealed that phosphorylation of UHRF1 S652 only leads to a modest (2-fold) reduction of the binding affinity of $UHRF1_{651-664}$ for USP7 (Figure S4A). Meanwhile, the serine-to-glutamate mutation of UHRF1 S664 (S664E), which is located immediately downstream of the USP7 interaction site (Figure 4A) and manifests M phase-specific

phosphorylation as well (Rigbolt et al., 2011), leads to a reduction of the UHRF1$_{647–687}$ binding affinity for USP7 by 4-fold (Figure S4B). Together, these observations suggest that multistep posttranslational modification events may be required to dissociate the UHRF1-USP7 complex during M phase.

Recent evidence has indicated that USP7 also deubiquitinates DNMT1 during S phase; in late S phase, acetylation of DNMT1 by Tip60 leads to disruption of the DNMT1-USP7 complex (Du et al., 2010). This previous work also prompted us to query whether UHRF1 can be acetylated by Tip60 at the PBR, which falls into the Tip60-preferred acetylation sequences (Kimura and Horikoshi, 1998). Indeed, following in vitro acetylation by recombinant Tip60, we detected acetylation of UHRF1 at K659 (Figure S4C) and found that this modification greatly decreased the UHRF1-USP7 interaction (Figure S4A), indicating a mechanism for UHRF1-USP7 dissociation. However, further studies are needed to define such a potential regulation of UHRF1 in vivo.

USP7 Interaction and Chromatin Targeting of UHRF1

One of the major findings in this study is that the USP7 interaction regulates the chromatin association of UHRF1. Consistent with this, a recent study showed that the UHRF1 S664E mutation, with a 4-fold reduction in USP7 binding based on our ITC analysis (Figure S4B), significantly reduced the chromatin binding of UHRF1 (Chu et al., 2012). Whereas the underlying mechanism of this regulation has yet to be elucidated, we show that the UHRF1-USP7 interaction interferes with the intramolecular TTD-PBR interaction of UHRF1, which therefore shifts the conformation of UHRF1 from the TTD-occluded state to a state that allows multivalent histone binding by the TTD-PHD. Such a change in the conformational dynamics of UHRF1 may synergize with the allosteric effect of PI5P (Gelato et al., 2014) in stabilizing the chromatin association of UHRF1. Indeed, a previous study showed that the nuclear level of PI5P is cell-cycle dependent, with ~20-fold enrichment in G1 phase (Clarke et al., 2001). However, the relationship between PI5P and the USP7-UHRF1 interaction remains to be determined. In addition, complex formation between UHRF1 and USP7 may also boost their respective interactions with the methyl-CpG-binding protein MBD4 (Meng et al., 2015), which has recently been shown to mediate the recruitment of USP7 to heterochromatin (Meng et al., 2015).

EXPERIMENTAL PROCEDURES

USP7 and UHRF1 proteins, except for the UHRF1$_{651–664}$ peptides, were expressed in BL21 (DE3) recombinant inbred line cells and purified through nickel affinity, size exclusion, and/or anion exchange chromatography. The crystal structure of the UHRF1$_{647–678}$-USP7 UBL$_{1–3}$ complex was determined by molecular replacement using the structure of the corresponding sequence of free USP7 (PDB: 2YLM) as the search model. ITC assays for USP7 and UHRF1 proteins were performed at 25°C. The resultant binding curves were processed with Origin 7.0 software (MicroCal). Chromatin association assays were performed in HeLa cells stably transfected with 3×Flag UHRF1 (wild-type and K657E/K659E) as previously described (Rothbart et al., 2013). Full details of experimental procedures are provided in Supplemental Experimental Procedures.

ACCESSION NUMBERS

The accession number for the structure of UHRF1-USP7 reported in this paper is PDB: 5C6D.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.07.046.

AUTHOR CONTRIBUTIONS

Z.-M.Z., S.B.R., D.F.A., J.S.H., Q.C., L.L., and J.S. performed the experiments. J.S., G.G.W., B.D.S., and Y.W. conceived the project. J.S. wrote the manuscript with the input of all the authors.

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