

STATE-OF-THE-ART REVIEW

Mechanism, specificity, and function of FANCD2-FANCI ubiquitination and deubiquitination

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Fanconi anemia (FA) is a rare genetic disorder caused by mutations in any of the currently 22 known FA genes. The products of these genes, along with other FA-associated proteins, participate in a biochemical pathway, known as the FA pathway. This pathway is responsible for the repair of DNA interstrand cross-links (ICL) and the maintenance of genomic stability in response to replication stress. At the center of the pathway is the monoubiquitination of two FA proteins, FANCD2 and FANCI, on two specific lysine residues. This is achieved by the combined action of the UBE2T ubiquitin-conjugating enzyme and a large multicomponent E3 ligase, known as the FA-core complex. This E2-E3 pair specifically targets the FANCI-FANCD2 heterodimer (ID2 complex) for ubiquitination on DNA. Deubiquitination of both FANCD2 and FANCI, which is also critical for ICL repair, is achieved by the USP1-UAF1 complex. Recent work suggests that FANCD2 ubiquitination transforms the ID2 complex into a sliding DNA clamp. Further ubiquitination on FANCI does not alter this closed-on-DNA ID2 conformation. However, the resulting dimonoubiquitinated complex is highly resistant to USP1-UAF1 deubiquitination. This review will provide an update on recent work focusing on how specificity in FANCD2 ubiquitination and deubiquitination is achieved. Recent findings shedding light to the mechanisms, molecular functions, and biological roles of FANCI/FANCD2 ubiquitination and deubiquitination will be also discussed.

Enzymes

UBA1 (6.2.1.45), UBE2T (2.3.2.23), FANCL (2.3.2.27), USP1 (3.4.19.12).

The Fanconi anemia pathway and FANCD2/FANCI ubiquitination

Fanconi anemia (FA) is a rare genetic disorder characterized by bone marrow failure, developmental abnormalities, and predisposition to cancer, while at the molecular level, FA cells are highly sensitive to agents (chemotherapeutic or endogenous aldehydes—primarily formaldehyde) that cause interstrand cross-links

(ICLs) in DNA. Such symptoms arise due to mutations (usually biallelic) in any of the 22 currently known Fanconi anemia complementation group (FANC) genes (A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q, R, S, T, U, V, and W). The proteins encoded by these FA genes, along with FA-associated

Abbreviations

AG20, FANCA-FANCG-FAAP20; BL100, FANCB-FANCL-FAAP100; CEF, FANCC-FANCE-FANCF; dsDNA, double-stranded DNA; ELF, E2-like fold; FA, Fanconi anemia; FAAP, Fanconi anemia-associated factor; ICL, interstrand cross-link; ID2, FANCI-FANCD2; RING, really interesting new gene; SIM, SUMO-interacting motif; SLD, SUMO-like domain; UAF1, USP1-associated factor 1; USP1, ubiquitin-specific protease 1.

proteins (FAAPs), participate in pathways that promote genomic integrity and/or prevent genomic instability, and the orchestrated action of these proteins is referred to as FA pathway [1–4].

A key step in the FA pathway is the monoubiquitination of FA proteins FANCD2 and FANCI. This is a crucial event because it results in the formation of FANCI-FANCD2 nuclear foci where numerous homologous recombination repair FA proteins are recruited. These proteins are involved in DNA incision, translesion synthesis, ICL removal, and break-induced replication and include BRCA2 (FANCD1), BRIP1 (FANCI), PALB2 (FANCN), RAD51C (FANCO), RAD51 (FANCR), BRCA1 (FANCS), XRCC2 (FANCU), XPF (FANCO), SLX4 (FANCP), REV7 (FANCV), and RFD3 (FANCW) [5]. The site-specific FANCD2 and FANCI ubiquitination occurs on two specific lysine residues (K561 and K523, respectively, for human proteins). This is achieved by the combined action of a large multicomponent E3 ubiquitin ligase, known as the FA-core complex, and the E2 ubiquitin-conjugating enzyme UBE2T, also known as FANCT (Fig. 1A). The FA-core complex comprises nine proteins organized in three submodules: BL100 (composed of two copies of FANCB, FANCL, and FAAP100), CEF (consisting of a single copy of FANCC, FANCE, and FANCF), and AG20 (made of two copies of FANCA and FANCG and 1 or 2 copies of FAAP20) [6,7] (Fig. 1B). The FA-core complex assembly is asymmetric, consisting of an active (FANCA', FANCG', FANCB', FANCL', and FAAP100') and an inactive (FANCA, FANCG, FAAP20, FANCB, FANCL, FAAP100, FANCC, FANCE, and FANCF) side (Fig. 1C). Of the three FA-core subcomplexes, BL100 is the most critical for FANCD2 ubiquitination, since depletion of either FANCB, FANCL, or FAAP100 completely abolishes FANCD2 ubiquitination *in vivo*, whereas BL100 complex can also support *in vitro* FANCD2 ubiquitination in the absence of the other two submodules [8]. The essentiality of BL100 for FANCI-FANCD2 *in vivo* ubiquitination is highlighted in recent FA-core structures, which indicate that BL100 most likely acts as an initial hub for FA-core assembly [7]. Importantly though, BL100 contains FANCL, a protein that possesses an E3 ubiquitin ligase activity, thanks to its C-terminal RING (Really Interesting New Gene) domain [9]. UBE2T, which transfers the ubiquitin to FANCD2/FANCI, binds directly to FANCL's RING domain [10,11]. The UBE2T-FANCL^{RING} interaction results in allosteric activation of UBE2T (Fig. 1D) for site-specific FANCD2 ubiquitination, and evidence suggests that FANCI site-specific ubiquitination is also achieved in the same manner [12].

The FANCI-FANCD2 (ID2) complex and the role of DNA in ID2 ubiquitination and deubiquitination

Both FANCD2 and FANCI are ubiquitinated during the S phase of cell cycle [13,14]. However, an increase in their ubiquitination and their colocalization at damage-induced nuclear foci is observed in response to DNA damage [14–16], as well as in response to replication stress [16,17]. Both FANCD2 and FANCI are deubiquitinated by the USP1-UAF1 complex [16,18–20], and USP1 deletion experiments in mice and in chicken cell lines have revealed that USP1-UAF1 activity is required for ICL repair and maintenance of genomic stability [21–23]. The genetic disruption of FANCI affects the stability, monoubiquitination, and chromatin localization of FANCD2, and of these, only the FANCD2 stability can be restored when an ubiquitin-deficient mutant of FANCI (K523R) is introduced. Conversely, replacement of wild-type FANCD2 with an ubiquitin-deficient mutant form (K561R) also abolishes both the monoubiquitination and chromatin localization of FANCI [14]. The above suggests that FANCD2 is in a constitutive complex with FANCI and that FANCD2 and FANCI monoubiquitination are interdependent. Accordingly, it is now well established that FANCD2 and FANCI assemble to form a complex, designated as the ID2 complex [14,24]. When in complex with FANCI, FANCD2's *in vitro* ubiquitination has been found to be greatly stimulated by various DNA structures [25–27], as well as by ssRNA and synthetic R-loop structures [28]. Furthermore, disruption of ID2 binding to DNA results in impaired FANCD2 ubiquitination [26,29]. Likewise, FANCI ubiquitination (which typically progresses at a much slower rate than FANCD2 ubiquitination) also gets greatly stimulated by DNA, whether this is in complex with FANCD2 or not [26,30]. The above suggests that upon FANCI/ID2 interaction with DNA or other nucleic acids, ubiquitinated FANCI (I_{Ub}) and two differentially ubiquitinated ID2 complexes ($I_{Ub}D2$ and $ID2_{Ub}$) may be initially generated for formation of an $I_{Ub}D2_{Ub}$ complex. The interaction of ubiquitinated ID2 complexes with DNA also seems to affect subsequent removal of ubiquitin from ubiquitinated ID2 complexes. Two studies have shown that dsDNA protects the $I_{Ub}D2_{Ub}$ complex from excessive USP1-UAF1 deubiquitination [20,27]. However, another study utilizing single-stranded DNA and partially ubiquitinated ID2 (approx. 60% FANCD2 ubiquitination and minimum/negligible FANCI ubiquitination) shows that DNA may be required for USP1-UAF1-mediated FANCD2 deubiquitination [31]. This finding

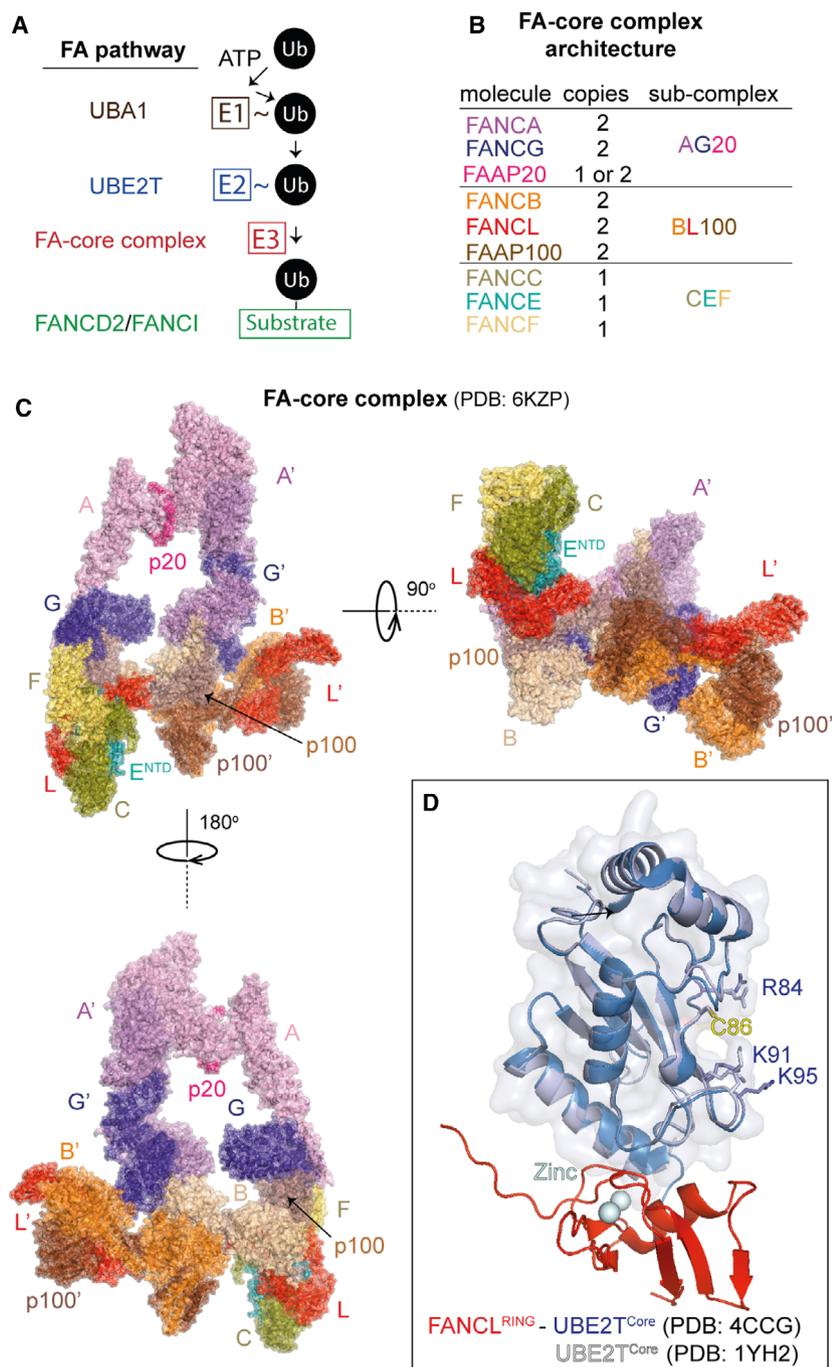


Fig. 1. The Fanconi anemia core (FA-core) complex and UBE2T. (A) Ubiquitination is a three-step enzymatic process, whereby ubiquitin is first activated by an E1 ubiquitin-activating enzyme (usually UBA1), with which it forms a thiol-ester bond (denoted as ~); then, it is transferred to the catalytic cysteine of an E2 ubiquitin-conjugating enzyme (again as a thiol-ester intermediate); finally, ubiquitin is transferred from the E2 enzyme to a lysine of a target protein with the help of an E3 ubiquitin ligase enzyme. Specificity is conferred by the E2-E3 pair. For FANCD2 and FANCI ubiquitination, UBE2T (also known as FANCT) is the E2 enzyme and the FA-core complex is the E3 enzyme. Whereas an ubiquitin chain can be formed by some E2-E3 pairs on target lysines, UBE2T and FA-core catalyze monoubiquitination reactions. (B) Subunit composition of the FA-core complex. Copies of each protein in the core and respective subcomplexes formed from these proteins are shown. (C) Human FA-core complex surface representation structure (PDB: 6KZP) [7]. The complex is shown at three different orientations with each FA protein labeled and shown in a unique color. Active site proteins are indicated with an apostrophe (') and are shown in different shades of the same color, apart from FANCL and FANCL' which are both colored red. The C terminus of FANCE is

disordered in the structure, and hence, only the N-terminal part of FANCE (E^{NTD}) is shown. (D) FANCL^{RING} binding to UBE2T's core/UBC (ubiquitin-conjugating) domain allosterically activates UBE2T, by reconfiguring the positions of three basic residues specifically targeting an acid patch on FANCD2 around its acceptor lysine (K561). Human UBE2T^{Core} (UBC domain only) apo structure (PDB: 1YH2) [82] was superimposed to human UBE2T^{Core} bound to the RING domain (residues 289–375) of FANCL (PDB: 4CCG) [11], using PyMOL. UBE2T's catalytic cysteine (C86), and the three basic residues (R84, K91, and K95) forming the basic triad are shown and illustrated as sticks.

contrasts with the observation that ID2_{Ub} complex can be efficiently deubiquitinated in both the presence and absence of DNA [20]. Thus, further studies are required to elucidate whether the type of DNA, differences in protein preparations including the potential for post-translational modifications, or other factors account for such discrepancies. A prerequisite for efficient *in vivo* ID2 ubiquitination is ATR-mediated FANCI phosphorylation on S556, S559, and S565 (human numbering) [14,32–36]. Recent biochemical data support the idea that these phosphorylation events have a dual role: They enhance ID2 ubiquitination by stabilizing the ID2 complex on DNA, while at the same time, they protect the complex from USP1-UAF1-mediated deubiquitination [36]. Interestingly though, USP1-UAF1 activity is not only affected by but is also required for optimal FANCI S559 and S565 phosphorylation [35], which suggests that a negative feedback mechanism may be in place to provide a balance between ubiquitination and deubiquitination. While all evidence suggests that FANCI phosphorylation favors the formation/maintenance of ubiquitinated ID2 complex, FANCD2 phosphorylation appears to disfavor this: *in vitro* and in-cell experiments with phospho-mimetic FANCD2 mutants, supporting the notion that, in the absence of DNA damage, ID2 recruitment to DNA and subsequent ubiquitination is inhibited via CK2-mediated FANCD2 phosphorylation [37].

The FANCD2-FANCI dimer (ID2 complex) has been shown to preferentially bind branched structures of DNA [38] and specifically ICLs [29,39]. A 7.8 Å crystallographic electron density map of FANCI bound to a splayed Y DNA provided insights into how this protein may bind branched DNA structures containing both double-stranded and single-stranded DNA [24]. While all ID2-dsDNA structures available show that only FANCI interacts with dsDNA, recent cryo-EM data of the ID2 complex bound to ICL DNA structure indicate that branching of the DNA facilitates a secondary, albeit less stable, DNA-ID2 interface between the branched dsDNA and FANCD2 (Fig. 2A), which likely contributes to the increased affinity of ID2 to branched, over nonbranched, DNA structures [39].

The lysines targeted for FANCD2 and FANCI monoubiquitination are within solvent-accessible tunnels of the ID2 interface [24,39] (Fig. 2A). While the lysines are not buried, they are inaccessible for UBE2T ubiquitination without ID2 conformational remodeling. Nevertheless, the presence of FANCI greatly stimulates FANCD2 ubiquitination (instead of restricting this), as shown in FANCL-catalyzed [12,25,40], BL100-CEF-catalyzed [27], and FA-core-catalyzed [6–8] FANCD2/ID2 ubiquitination reactions. Moreover, FANCI further restricts the site of FANCD2 ubiquitination to the physiologically modified lysine of the latter [40]. Since DNA can stimulate FANCD2 *in vitro* monoubiquitination within the ID2 complex [6,12,25–27,29], irrespective of the source of E3 ligase used (FANCL, BL100-CEF, or FA-core complex), it has been suggested that DNA may be responsible for remodeling the ID2 complex to allow access for the E2/E3 machinery [25]. Nevertheless, no major ID2 conformational changes have been identified upon DNA binding, based on the highly similar cryo-EM structures of apo-ID2 complex and the ICL-bound ID2 complex [39]. FANCL, along with various components of the FA-core complex and ubiquitin-charged UBE2T, is hence assumed to promote such conformational change, once ID2 is bound to the DNA. Indeed, that is what recent cryo-EM structures of FA-core-UBE2T-ID2-DNA complexes suggest [7] (Figs 2B and 3B). Moreover, *in vitro* studies have shown that, while FANCL and BL100 have comparable E3 activities against isolated FANCD2 (whose ubiquitination does not require DNA) [12], BL100 (and/or BL100-CEF) along with DNA is required for efficient ubiquitination of the ID2 complex [8,27]. The FA-core-UBE2T architecture and the contribution of each of the FA-core-UBE2T components for site-specific ID2 ubiquitination will be further discussed below.

The role of UBE2T and the FA-core complex in ID2 ubiquitination

As mentioned above, UBE2T coupled with BL100 can support *in vitro* ID2 ubiquitination, in the absence of other FA-core subunits, which indicates that AG20 and CEF play an auxiliary to BL100 function. A FANCA asymmetric dimer is presumably required for

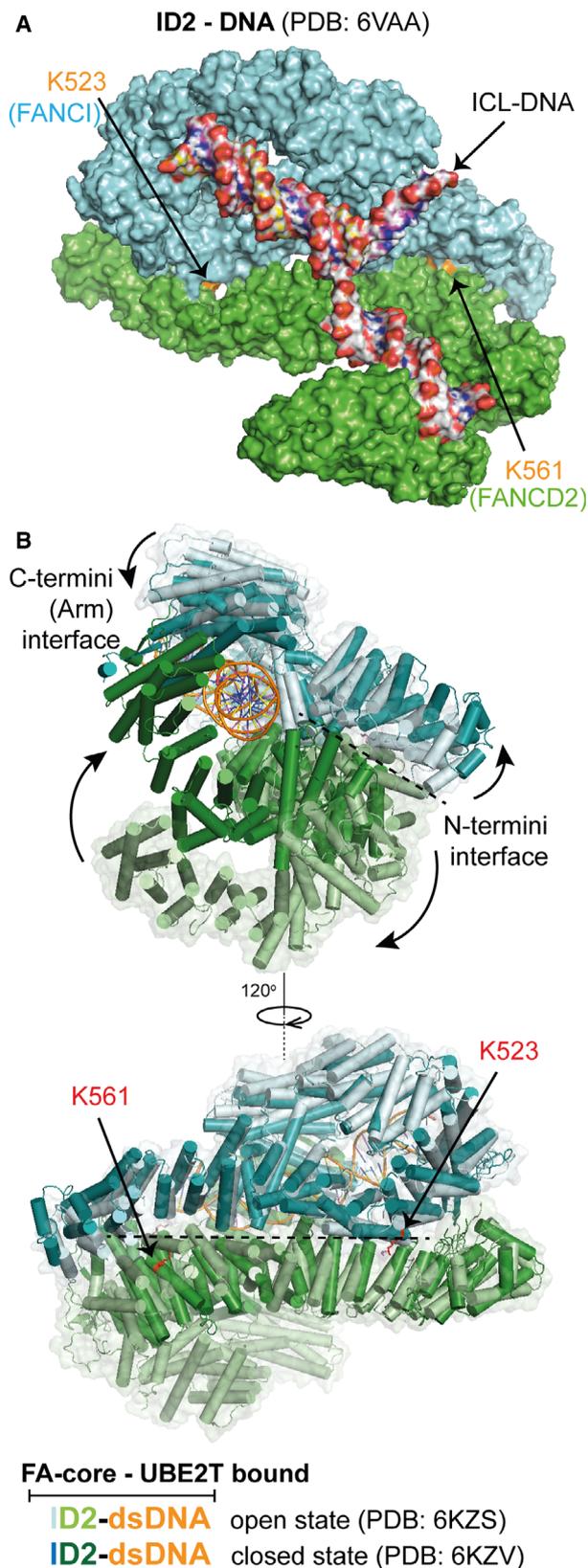


Fig. 2. The target lysines of FANCD2 and FANCI are inaccessible for ubiquitination and thus require remodeling of the ID2 structure. For FANCD2 ubiquitination, this is achieved upon binding of ID2 to FA-core, UBE2T, and DNA. (A) Position of target ubiquitination lysines in nonubiquitinated ID2 complex bound to interstrand cross-linked (ICL) DNA (PDB: 6VAA; surface representation) [39]. (B) ID2 complex bound to DNA, FA-core, and ubiquitin-charged UBE2T, transitions between two conformations (open and closed), prior to FANCD2 ubiquitination. The closed-state structure of ID2-dsDNA (cartoon representation; PDB: 6KZV [7] with FA-core and UBE2T removed) was superimposed on the open-state ID2-dsDNA structure (cartoon and surface representation; PDB: 6KZS [7] with FA-core and UBE2T removed and dsDNA not shown) using PyMOL. Movements of FANCI and FANCD2 chains are shown upon transition from the open to the closed state. The transition involves movement of the C termini of FANCI and FANCD2, which interact (Arm ID2 interface) and encircle double-stranded DNA (dsDNA) in the closed state, whereas the N-termini ID2 interface (dotted black line) partially loosens to expose K561 of FANCD2, and to a lesser extent K523 of FANCI. Both lysines are shown as red sticks in the closed-state ID2 structure and as pink sticks in the open-state ID2 structure.

the assembly of an active asymmetric FA-core complex. In support for this, a large proportion of FA pathological missense mutations are located within FANCA dimerization domains; moreover, lack of FANCA dimerization is associated with assembly of a twofold symmetric FA-core complex, which is predicted to be inactive [7]. FANCA dimerization occurs via the C termini of two FANCA molecules [7,41], whereas the two FANCG molecules bridge the FANCA dimer (on which FAAP20 also binds) to the dimeric BL100 module [7] (Fig. 1C). BL100, which is a dimer of trimers, composed of two copies of FANCB, FANCL, and FAAP100 [6,7,27,42], and bridges the dimeric AG20 module to a single copy of CEF [7]. The above interactions result in an asymmetric FA-core complex, with an active side primed for UBE2T and ID2 binding [7] (Figs 1C and 3A–C). The structural configuration of the asymmetric FA-core complex enables the active side FANCL (FANCL') and a C-terminal domain (CTD) of FANCE (FANCE_{CTD}, which is flexible in the absence of ID2) to bind and remodel ID2-DNA for FANCD2 ubiquitination, whereas the inactive (within FA-core) FANCL binds the N-terminal segment of FANCE, along with FANCC and FANCF [7] (Figs 1C and 3A–C). The asymmetry might be crucial to ensure that only one copy of CEF complex will be bound to the BL100 module, thus allowing (the active side) FANCL' to engage with FANCE_{CTD} and UBE2T for ID2 remodeling and ubiquitination [7] (Fig. 3A,B). Nevertheless, addition of the AG20 module in BL100-CEF-catalyzed

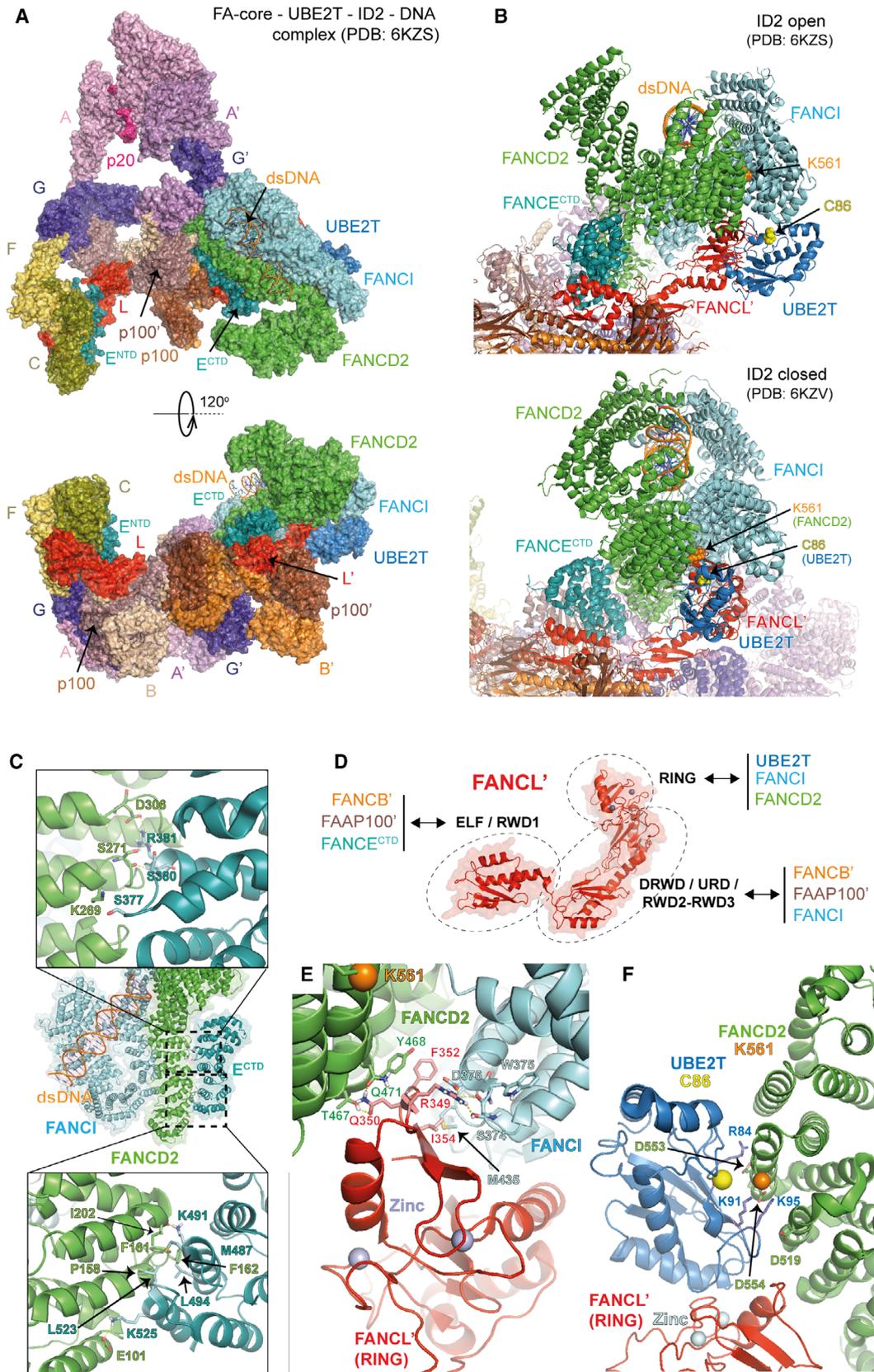


Fig. 3. Structural basis of FANCD2 ubiquitination by the FA-core and UBE2T. During FA-core-catalyzed FANCD2 ubiquitination, a C-terminal segment of FANCE (E^{CTD}) binds to FANCD2, while FANCL inserts into the ID2 interface in proximity to FANCD2' K561. The above interactions, along with FANCI-binding to dsDNA, result in ID2 transitioning from an open- to a closed-state conformation. In the closed-state ID2 structure, UBE2T is able to engage with FANCD2. As a result, the cysteine of UBE2T carrying the ubiquitin (C86) is positioned within proximity to the target lysine of FANCD2 (K561) for ubiquitin transfer. (A) Surface representation of FA-core-UBE2T bound to ID2-dsDNA (open-state ID2; PDB: 6KZS) [7]. The overall complex is shown at two orientations with each FA protein labeled and shown in a unique color. Active side proteins are indicated with an apostrophe (') and are shown in different shades of the same color, apart from FANCL and FANCL' that are both colored red. (B) Cartoon representation of FA-core-UBE2T bound to ID2-dsDNA, centered on FANCL-FANCD2, in both open-state ID2 (PDB: 6KZS; *top*) and closed-state ID2 (PDB: 6KZV; *bottom*) structures [7]. Views along the DNA axis are shown for both structures. Labeling and coloring are as in A. The positions of the UBE2T cysteine carrying the ubiquitin (C86; yellow spheres) and of the target FANCD2 lysine (K561; orange spheres) are shown in both conformations. (C) Close-up view of FANCE E^{CTD} - FANCD2 interactions in open-state ID2 conformation (PDB: 6KZS). Residues predicted to be involved in polar and hydrophobic interactions for each protein are shown as sticks and labeled. Hydrogen bonds are shown as yellow dotted lines. For clarity, all other elements of the FA-core and UBE2T were removed. (D) Surface, cartoon representation, domain architecture, and protein interactions of active side FANCL (FANCL') in FA-core-UBE2T-ID2-dsDNA structure (PDB: 6KZS). (E) Close-up view of FANCL' RING insertion into the ID2 interface (PDB: 6KZS). For clarity, UBE2T and some elements of FANCI were removed. Proteins are shown as cartoons, the epsilon-amino group of FANCD2's target lysine (K561) is shown as orange sphere, and residues predicted to participate in hydrogen bond (dotted yellow lines), and hydrophobic interactions in the structure are shown as sticks. (F) Close-up view of UBE2T engaging FANCD2 for ubiquitination, in the closed-state ID2 conformation (PDB: 6KZS). For clarity, among FA-core proteins, only the RING domain of FANCL' is shown. Structures are illustrated as cartoons. The basic residues forming the catalytic triad of UBE2T (R84, K91 and K95) and the acidic patch aspartate residues of FANCD2 (D519, D553, and D554), which are recognized by UBE2T's basic triad are shown and illustrated as sticks. UBE2T's cysteine carrying the ubiquitin (C86; yellow) and FANCD2's target lysine (K561; orange) are illustrated as spheres.

ID2 ubiquitination reactions has not been found able to further improve FANCD2/FANCI *in vitro* ubiquitination [27]. Furthermore, the reaction rate of ID2 ubiquitination with chicken proteins is comparable between BL100 and FA-core-catalyzed reactions [6,8]. The above suggests that AG20 is not directly involved in catalysis. On the other hand, human CEF has been reported to significantly enhance *in vitro* BL100-catalyzed ubiquitination of FANCD2 and FANCI, but only when these proteins exist in the ID2 heterodimer [27]. However, this function may not be very well conserved among vertebrates, since CEF appears to be dispensable for maximal ID2 ubiquitination in *in vitro* reactions with chicken proteins [6,8]. Earlier reports have shown that CEF is required for recruiting the BL100 unit to sites of DNA damage [43] and for bridging the ID2 complex to BL100 [27]. In a nonredundant fashion, the AG20 module also works to recruit BL100 to sites of DNA damage, but independently of CEF: Simultaneous knockdown of both FANCG and FANCF results in a complete abolishment of FA-core complex chromatin recruitment and FANCD2 monoubiquitination; however, single deletion of FANCG or FANCF does not [43]. As mentioned above, FANCE E^{CTD} (residues 273–536) interacts with the N terminus (residues 91–311) of FANCD2, acting thus as a substrate adaptor protein [7,44,45] (Fig. 3C). This interaction has been additionally proposed to result in a reshaped, more stable ID2 interface [42]. Upon FANCE E^{CTD} -FANCD2 binding, a

rotation of a ~ 160 amino acid segment of FANCD2 occurs, contributing to partial exposure of FANCD2's K561 [7]. Recently, it has been shown that CEF can also bind to FANCI, in the absence of FANCD2 [27]. However, it remains to be established what function this interaction may serve, since no FANCE-FANCI interaction has been observed upon FANCD2 ubiquitination [7].

For site-specific FANCD2/FANCI ubiquitination, FANCL is the RING E3 ligase, which binds and activates UBE2T for site-specific FANCD2/FANCI ubiquitination. The crystal structure of the drosophila full-length FANCL revealed that this protein contains, apart from a RING domain, also an ELF (E2-Like Fold), as well as a DRWD (Double RWD) domain (Fig. 3D); the latter was found capable of binding both FANCI and FANCD2 [46]. The equivalent DRWD domain in human FANCL was later crystallized and designated as URD (UBC-RWD Domain). This has a similar structure to the drosophila FANCL DRWD (despite differing significantly in amino acid conservation) and likewise binds both FANCD2 and FANCI substrates *in vitro* [47]. Indeed, FANCL's URD, also referred to as RWD2-RWD3 domain, has been shown to bind FANCI, in recent cryo-EM FA-core-UBE2T-ID2 structures, captured prior to FANCD2 ubiquitination [7]. The main function of the ELF domain, designated also as RWD1 in human FANCL, appears to be in binding FANCB-FAAP100 for formation of a BL100 complex [7,27,42]. However,

in active side FANCL (FANCL') it additionally interacts with the C-terminal domain of FANCE, which in turn binds to FANCD2 [7] (Fig. 3A,B,D). Accordingly, an intact ELF is essential for *in vivo* FANCD2 ubiquitination [48]. The ELF domain of FANCL is also capable of binding ubiquitin's Ile44 patch [48], but it is currently unknown whether there is a FANCL-ubiquitin-binding step during ID2 ubiquitination. FANCL's RING domain plays role in ID2 remodeling prior to FANCD2 ubiquitination; a hairpin of FANCL' RING domain inserts into the ID2 interface in proximity to FANCD2's K561, interacting with both FANCD2 and FANCI (Fig. 3E), as demonstrated in a recent cryo-EM structure of ID2 bound to FA-core complex [7]. Crucially though, the RING domain of FANCL binds and recruits UBE2T with high specificity (Figs 1D and 3F). This is achieved by a combination of electrostatic and hydrophobic interactions, which are unique for the UBE2T-FANCL^{RING} pair [11].

FANCL's RING interaction with UBE2T optimizes several uniquely positioned positively charged residues within UBE2T (Fig. 1D) to drive specific lysine selection on FANCD2 (K561) via recognition of a conserved negatively charged patch adjacent K561 [7]. Indeed, the importance of these interactions for FANCD2 ubiquitination has been demonstrated with charge-reversal mutations on UBE2T's catalytic basic triad. Such mutations impair FANCD2 ubiquitination, but FANCD2 ubiquitination can be restored if reverse-charge mutations are also applied on FANCD2's acidic patch [12]. Moreover, these acidic residues are aligned within proximity to UBE2T basic residues prior to ubiquitin transfer, in a recent cryo-EM FA-core-UBE2T-ID2-DNA structure [7] (Fig. 3F). Lastly, targeted mutagenesis on UBE2T (E54R, P93G, and P94G mutations), based on the allosteric conduit identified, can also result in optimization of UBE2T's charged residues for enhanced FANCD2 ubiquitination (in both the presence and the absence of FANCL), without compromising site specificity [12]. UBE2T contains, apart from its conserved core (UBC) domain, also a ~40 amino acid C-terminal extension appended to this. Although it is unclear whether this region has any role in FANCI ubiquitination, all available evidence suggests that this is dispensable for FANCD2 ubiquitination. Previous studies have shown that UBE2T is ubiquitinated on both K91 and that C-terminal extension [10,40], but a UBE2T mutant lacking these sites (UBE2T-ΔC-K91R) efficiently supports FANCD2 ubiquitination, both *in vitro* and *in vivo* [40]. Moreover, no density for this region has been identified in recent cryo-EM FA-core-UBE2T-ID2-dsDNA structures [7].

As mentioned above, BL100 results in more efficient ID2 ubiquitination than FANCL alone. Although it is currently unclear why this occurs, it is likely that FANCL is more stable within a BL100 complex. FANCB is responsible for dimerization of the BL100 complex, while FAAP100, which adopts a similar to FANCB fold, most likely stabilizes FANCB (and hence the dimeric BL100 complex) and is additionally required for bridging BL100 to AG20 [6,7,27,42]. However, the recruitment of two FANCL molecules by the FANCB-FAAP100 tetramer does not result in simultaneous ubiquitination of FANCD2 and FANCI within an ID2 complex, as previously hypothesized [42]. The BL100 dimeric architecture within the FA-core is not fully symmetric, but instead adopts a pseudo twofold symmetry [6,7]. Furthermore, several studies point to the fact that FANCD2 is the preferred substrate of ubiquitination within the ID2 complex [7,14,25–27,29]. However, in the absence of FANCI, FANCD2 is a poor substrate for FA-core-catalyzed ubiquitination [6–8]. This may be due to FANCD2's ability to form homodimers, in which the target lysine is buried in the FANCD2-FANCD2 interface, as indicated in a recent cryo-EM structure of recombinant chicken FANCD2, coupled with size-exclusion FANCD2/FANCI/ID2 experiments [49]. Alternatively (or additionally), FANCI's interaction with FANCL and/or UBE2T [7] may be required for efficient targeting of FANCD2's K561 by the FA-core complex.

While the FA-core complex with UBE2T seems to specifically open the ID2 dimer for FANCD2 ubiquitination [7], FANCD2 ubiquitination is required for efficient FANCI's ubiquitination. Indeed, substitution of FANCD2 with a nonubiquitinatable lysine-to-arginine mutant results in complete loss of FANCI ubiquitination in *in vitro* BL100- or BL100-CEF-catalyzed ID2 ubiquitination reactions [27]. Furthermore, in an *in vitro* setting with genetically engineered UBE2T, FANCI, and FANCD2 proteins, 'switching off' only FANCD2 ubiquitination results in decreased FANCI ubiquitination, while 'switching back on' FANCD2 ubiquitination significantly restores FANCI ubiquitination [12]. Recent cryo-EM structures of FANCD2-ubiquitinated ID2 complex have shed light into how FANCD2 ubiquitination can enhance FANCI ubiquitination: ubiquitin conjugation on FANCD2's lysine 561 is associated with major FANCD2 conformational changes [30,39,49], resulting in exposing FANCI's lysine 523 for ubiquitination [39] (Fig. 4). This lysine is likely targeted by UBE2T by a similar allosteric mechanism of FANCL-mediated UBE2T activation [12]. However, it remains to be determined how FA-

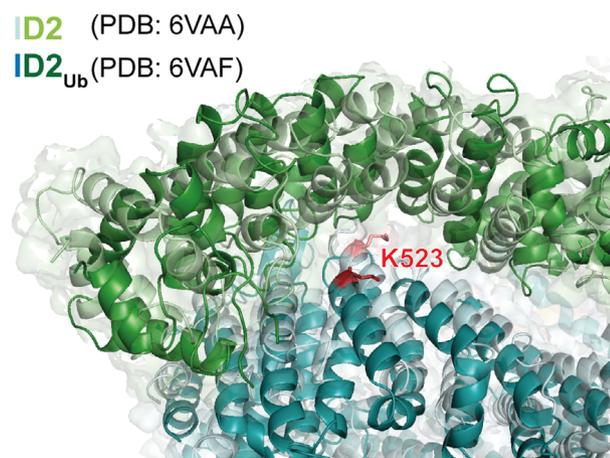


Fig. 4. FANCD2 ubiquitination leads to structural rearrangements in the ID2 complex, resulting in K523 of FANCI being more accessible for ubiquitination. Structural changes upon FANCD2 ubiquitination occurring in the ID2 complex surrounding K523 of FANCI. The FANCI-FANCD2-DNA complex structure (PDB: 6VAA [39]; displayed as cartoon and surface) was superimposed to FANCI-FANCD2_{Ub}-DNA complex (PDB: 6VAF [39]; shown as cartoon only), and centered on K523 of FANCI (colored as pink in 6VAA and dark red in 6VAF) using PyMOL. The superimposition of the two structures shows that FANCD2 helices move away from FANCI's K523, while the FANCI helix containing K523 is also shifted away from FANCD2.

core and UBE2T specifically engage with ID2_{Ub} (or ID2) for FANCI ubiquitination. Interestingly, and contrary to human FANCD2, human FANCI in isolation can be efficiently ubiquitinated by the FA-core complex [7]. The nearly 10-fold excess protein abundance of FANCI over FANCD2, reported in U2OS cells [50], indicates that FANCI may be ubiquitinated in isolation to some extent in cells too. However, a substantial fraction of cellular FANCI may not be available for ID2 formation and ubiquitination. Apart from participating in the FA pathway, FANCI also functions in ribosome biogenesis in the nucleolus and such function requires neither FANCD2 nor ubiquitination [51]. Furthermore, all available *in vivo* data suggest that the presence of ubiquitinatable FANCD2 is required for efficient FANCI ubiquitination: Decreased FANCI protein levels and undetectable FANCI ubiquitination have been reported in the absence of cellular FANCD2, whereas introduction of a K561 FANCD2 mutant to these cells could restore only the FANCI cellular levels, but not FANCI ubiquitination [14]. Hence, FANCD2 ubiquitination is required for initiation and/or maintenance of FANCI ubiquitination.

The molecular function of FANCD2 and FANCI ubiquitination

Although it has been known since 2001 that FANCD2 becomes ubiquitinated in response to DNA damage, the molecular function of this modification had been elusive for nearly two decades. Ubiquitination of the ID2 complex has been long hypothesized to result in recruitment of ubiquitin-binding factors, essential for ICL repair or restoration of replication. Ubiquitin-binding proteins typically recognize a hydrophobic patch on ubiquitin that includes Leu8, Ile44, and Val70 [52]. However, the recent cryo-EM structure of human dimonoubiquitinated ID2 complex bound to DNA (I_{Ub}D2_{Ub}-DNA) revealed that both conjugated ubiquitins have this hydrophobic patch buried within the I_{Ub}D2_{Ub} interface [39] and are thus not easily accessible to ubiquitin-binding proteins. Indeed, another study utilizing pull-down binding experiments showed that ID2 ubiquitination had no effect on ID2 interactions with several DNA-repair proteins that contain ubiquitin-binding domains [53]. Three independent studies have now shown that upon FANCD2 ubiquitination, major FANCD2 conformational changes occur, resulting in a newly formed ID2 interface, formed by the two C-terminal 'arms' of FANCI and FANCD2 (Arm interface), and a monoubiquitinated ID2 complex (ID2_{Ub}) encircling double-stranded DNA (dsDNA) [30,39,49] (Fig. 5). The resulting ID2_{Ub} complex has significantly increased affinity for dsDNA, compared with the nonubiquitinated ID2 complex [30,49]. Further ubiquitination on FANCI does not seem to impact on the overall ID2_{Ub} structure and coupled DNA affinity, since the dimonoubiquitinated (I_{Ub}D2_{Ub}) complex also displays a comparable dsDNA affinity and overall ID2 conformation to the ID2_{Ub} complex [30,39] (Fig. 5). No specificity for a particular DNA sequence has been reported for ubiquitinated/nonubiquitinated ID2. Accordingly, in all available ubiquitinated ID2-dsDNA cryo-EM maps, the dsDNA is less well resolved, which indicates that dsDNA populates multiple positions/orientations upon ID2 binding. Furthermore, structural evidence suggests that the I_{Ub}D2_{Ub} complex is able to bind only to a dsDNA stretch (of about 30 kb) of various DNA structures, due to electrostatic interactions occurring between a semicircular I_{Ub}D2_{Ub} basic groove and the acidic DNA backbone [39]. The above suggests that ubiquitinated ID2 may act as a sliding DNA clamp. Indeed, evidence indicates that the I_{Ub}D2_{Ub} complex is able to slide on dsDNA, since it can come off a linear stretch of dsDNA, but cannot disengage from a circular dsDNA of the same length [39]. DNA sliding has been suggested to enable the ID2 complex to travel away

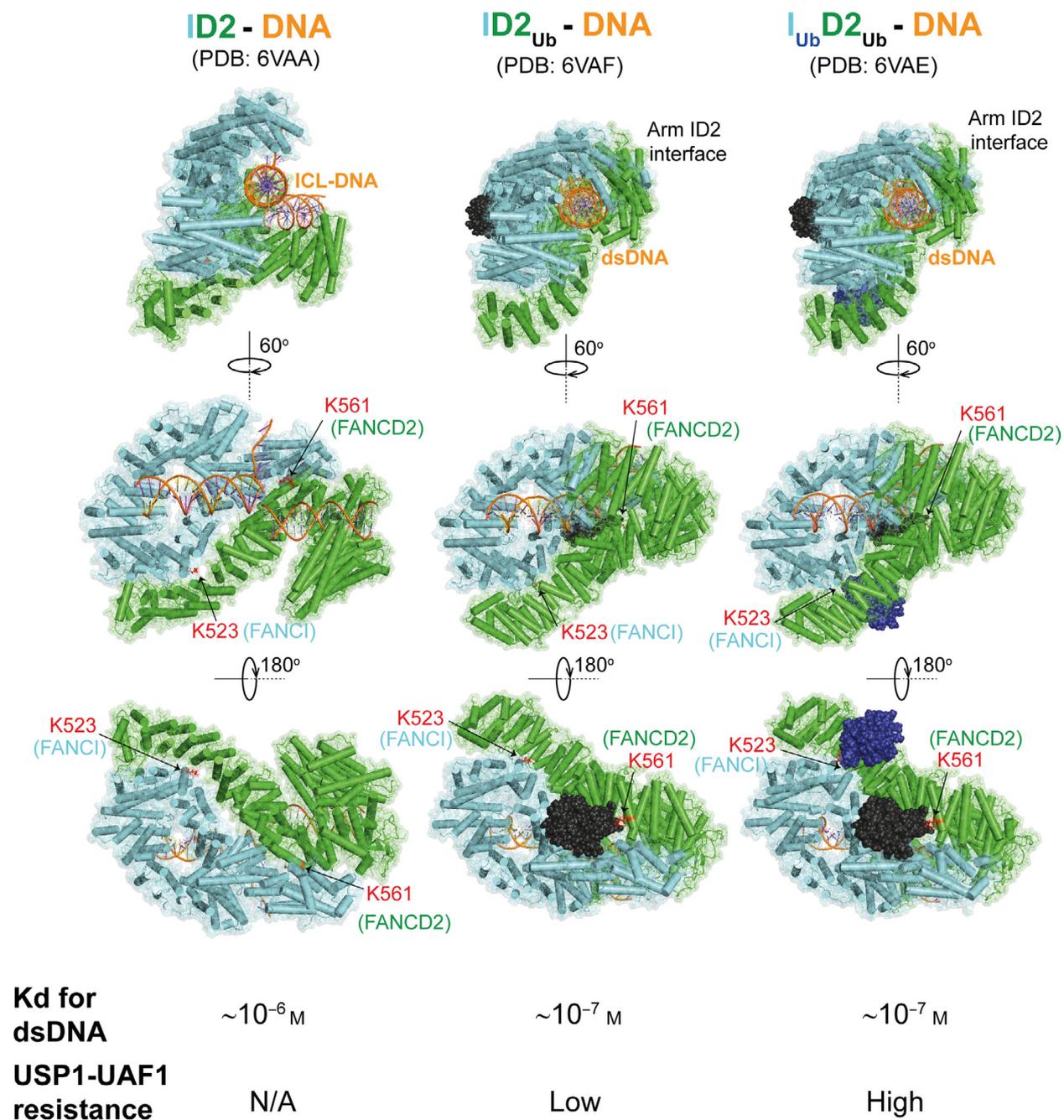


Fig. 5. FANCD2 ubiquitination transforms the ID2 complex into a DNA clamp, whereas further ubiquitination on FANCI locks this conformation by conferring resistance to USP1-UAF1-mediated FANCD2 deubiquitination. Cartoon and surface representation of human: nonubiquitinated FANCI-FANCD2 (ID2) complex bound to an interstrand cross-linked (ICL) DNA (ID2-DNA; PDB: 6VAA); FANCD2-ubiquitinated ID2 complex bound to double-stranded (ds) DNA (ID2_{Ub}-DNA; PDB: 6VAF); and FANCD2- and FANCI-ubiquitinated ID2 complex bound to dsDNA (I_{Ub}D2_{Ub}-DNA; PDB: 6VAE) [39]. Each complex is shown at three different angles. FANCD2 ubiquitination stabilizes a secondary FANCD2-FANCI interface formed between the C-terminal ‘arms’ of each protein (Arm ID2 interface), resulting in the ID2 complex encircling dsDNA. The FANCD2 and FANCI lysines that get modified (K561 and K523, respectively) are indicated in red. FANCD2 (green), FANCI (cyan), DNA backbone (orange), ubiquitin conjugated to FANCD2 (black), and ubiquitin conjugated to FANCI (dark blue). The dissociation constants (Kd values) of each complex for dsDNA in solution, as calculated from protein-induced fluorescence enhancement (PIFE) data [30], are shown. The relative resistance (low/high) of FANCD2’s ubiquitin to USP1-UAF1 deubiquitination activity is also indicated for mono (ID2_{Ub}-DNA) and dimonoubiquitinated (I_{Ub}D2_{Ub}-DNA) ID2 complexes.

from ICLs, replication forks, or other related DNA structures, on which ID2 ubiquitination has occurred [39]. *In vitro* studies have shown that, upon ubiquitination, multiple ID2 complexes can accumulate on long stretches of dsDNA (150 bp or longer), forming filament-like structures; the latter have been suggested to act as a DNA-shield, protecting the free dsDNA from the unwanted action of nucleases, helicases, or other factors [53]. Indeed, FANCD2 ubiquitination has previously been shown to protect stalled replication forks from degradation [54]. However, it is not yet known whether ID2 complexes form such filament-like structures *in vivo*.

The fact that FANCI ubiquitination does not further change the overall structure and associated dsDNA affinity of the ID2_{Ub} complex suggests that FANCI ubiquitination may have a complementary function to FANCD2 ubiquitination. Indeed, *in vitro* deubiquitination experiments with USP1-UAF1 and either ID2_{Ub}-DNA or I_{Ub}D2_{Ub}-DNA substrates have shown that FANCI ubiquitination largely protects FANCD2's ubiquitin from USP1-UAF1 activity [30]. This may also explain why introducing a nonubiquitinatable FANCI mutant (K523R) in FANCD2 ubiquitination-proficient cells results in greatly reduced FANCD2 ubiquitination [14]: The ubiquitin conjugated to FANCD2 would not be sufficiently protected against USP1-UAF1 activity, when the FANCD2 forms a complex with FANCI^{K523R}. Key for this protection appears to be FANCI's ubiquitin interaction with FANCD2, which involves the Ile44 hydrophobic patch of ubiquitin [39]. Indeed, I44 alanine substitution of FANCI's conjugated ubiquitin makes FANCD2's ubiquitin more susceptible to USP1-UAF1 activity [30]. In the I_{Ub}D2_{Ub}-DNA complex, FANCI's ubiquitin is also partially protected from USP1-UAF1 activity, since FANCI's deubiquitination appears to be even slower than FANCD2's [27,30]. Although the precise reason for this is currently unknown, the I_{Ub}D2_{Ub}-DNA complex structure suggests that the ubiquitin conjugated to FANCD2 is less buried than the ubiquitin conjugated to FANCI [39,55] and hence may be more accessible for USP1-UAF1 deubiquitination. The following section will focus on how USP1-UAF1 interacts with the FANCD2-ubiquitinated ID2 complex for FANCD2 deubiquitination.

Mechanism of FANCD2 deubiquitination

As previously mentioned, FANCI/FANCD2 deubiquitination is specifically catalyzed by the USP1-UAF1 complex. USP1, which belongs to the ubiquitin-specific protease (USP) class of deubiquitinating enzymes, is the

subunit binding to ubiquitin and catalyzing its removal from (the conjugated with that) proteins. As revealed in recent crystal and cryo-EM structures of USP1 [55], this has a typical USP-fold, consisting of Fingers, Palm, and Thumb subdomains [56]. Apart from its USP-core domain, USP1 also has two insertions and an N-terminal extension appended to this domain. On its own, USP1 has little activity and hence requires activation by UAF1 (USP1 Associated Factor 1; also known as WDR48) for efficient catalytic turnover [19]. UAF1, which can also form a complex with and activate USP12 and USP46 [57], binds and activates all USP partners via its WD40-repeat β -propeller domain [19,57]. This domain specifically interacts with the finger domain of USPs, as demonstrated in recent UAF1-USP12 [58,59], UAF1-USP46 [60], and UAF1-USP1 [55] structures. While USP12 and USP46 also require a second activator (WDR20) for maximal activity, USP1 does not [61,62]. However, recent work has indicated that part of USP1's low intrinsic activity is due to autoinhibition conferred by the two insertions to its USP domain, which is alleviated upon binding to UAF1 [63]. A 54 amino acid segment within USP1's N-terminal extension is required for efficient cleavage of ubiquitin from FANCD2's K561, but is dispensable for FANCI's K523 ubiquitin removal [20].

Although limited information exists on the role of UAF1's Ancillary domain, the SUMO-like domain (SLD) of UAF1 has been proposed to bind a putative SUMO-interacting motif (SIM) on FANCI, and a SIM peptide sequence on the PCNA-binding protein hELG1. Accordingly, replacement of wild-type UAF1 with a mutant UAF1 version lacking part of its SLD, in chicken DT40 cells, results in impaired FANCD2 and PCNA deubiquitination [64].

A recent cryo-EM structure of a human USP1-UAF1-ID2_{Ub}-DNA complex has provided insights into how the USP1-UAF1 complex deubiquitinates FANCD2 [55]. This structure has revealed that USP1 extracts the ubiquitin from the FANCD2_{Ub}-FANCI interface, to enclose it into USP1's Palm and Thumb USP subdomains. As a result, the ubiquitin is 160° rotated relative to its position in the ID2_{Ub}-DNA complex (without USP1-UAF1), and the isopeptide bond between K561 of FANCD2 and G76 of ubiquitin is in proximity to USP1's active site C90 for cleavage (Fig. 6A). The structure additionally revealed two important UAF1-FANCI interfaces: UAF1's SUMO-like domain (SLD) interacting with FANCI's 249-260 amino acid loop (Fig. 6A,B) and UAF1's β -propeller interacting with FANCI's 547-576 amino acid loop (Fig. 6A,C). The importance of these interactions is supported by associated biochemical data, whereby

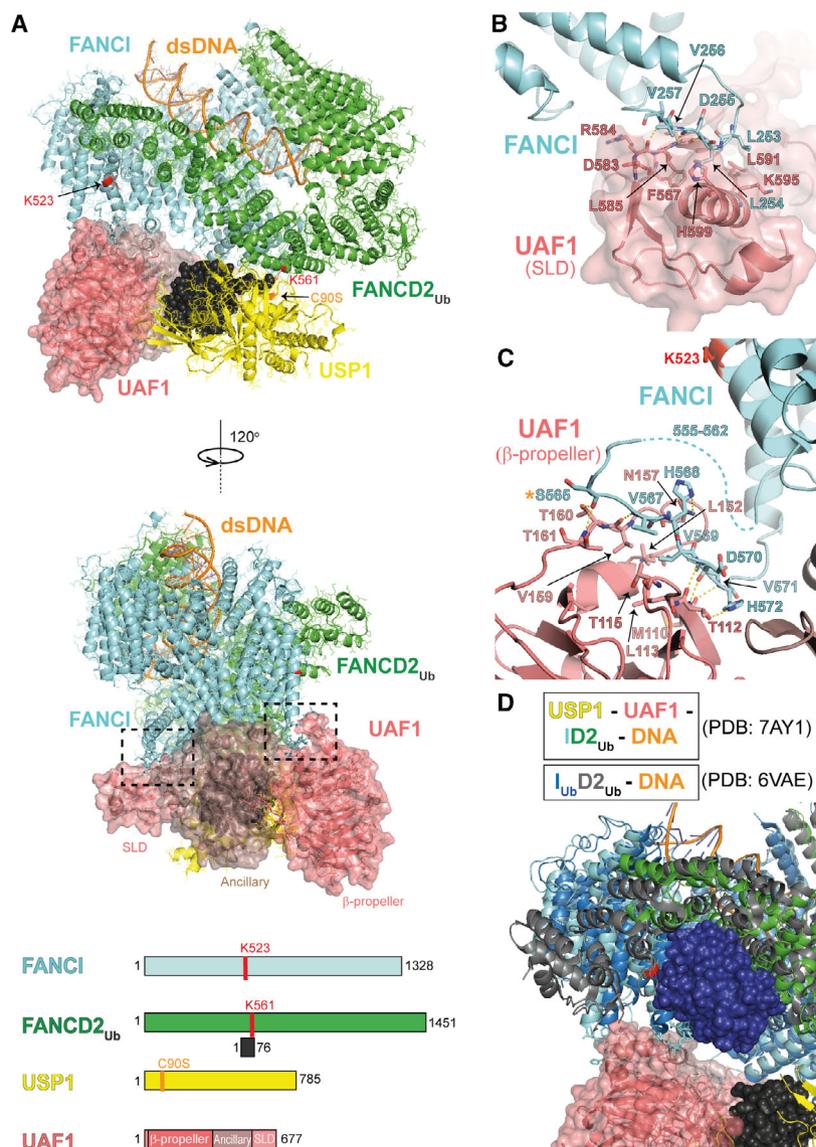


Fig. 6. Structural insights into FANCD2 deubiquitination by USP1-UAF1. (A) cryo-EM structure of a human USP1-UAF1-FANCI-FANCD2_{Ub}-dsDNA complex (PDB: 6AY1) [55]. *Top*: FANCD2 (green), FANCI (cyan), USP1 (C90S mutant; yellow), and DNA (orange backbone) are illustrated as cartoons, ubiquitin (black) as spheres, whereas the three domains of UAF1: β-propeller (deep salmon), Ancillary (dark salmon), and SUMO-like domain (SLD; salmon) are shown as both cartoons and surfaces. *Middle*: same as top but rotated 120° anticlockwise around y-axis. Dashed squares show the regions of FANCI interaction with UAF1. *Bottom*: schematic diagram of human proteins depicted in cryo-EM structure, with location of key features in their respective amino acid chain shown. (B, C) Close-up view of FANCI's interaction with the SLD domain (B) and β-propeller domain (C) of UAF1. For clarity, only FANCI and UAF1 from the USP1-UAF1-ID_{Ub}-DNA structure are displayed. Residues predicted from the structure to engage in hydrophobic or polar interactions (dashed yellow lines) are shown as sticks. From those, the ones that likely interact using their side chain are labeled (cyan for FANCI and salmon for UAF1). A part of FANCI's 547–576 amino acid segment (in proximity to UAF1's β-propeller) not modeled in the structure (residues 555–562) due to poor density, is shown as dotted line. S565 (also displayed, labeled and highlighted with an orange asterisk) has been previously shown to be phosphorylated in an ATR-dependent way. (D) USP1-UAF1 complex likely engages the I_{Ub}D2_{Ub} complex in the same way as the ID_{Ub} complex (for FANCD2 deubiquitination), since superimposition of I_{Ub}D2_{Ub}-DNA structure (PDB: 6VAE) [39] to the USP1-UAF1-ID_{Ub}-DNA structure (PDB: 6AY1) [55] does not reveal any clashes of FANCI's ubiquitin (blue spheres) with UAF1.

deletion of UAF1's SLD or phospho-mimetic mutations on FANCI at FANCI-β-propeller interface impairs FANCD2 deubiquitination [55]. The SIM-like

sequence of FANCI that interacts with UAF1's SLD (DELLDVV) is different and far away from the one previously postulated (VIPLQQGEEEEEEEE) [64]. It

is thus unclear how deleting part of the latter sequence results in increased levels of ubiquitinated FANCD2 in cells [64], since this has shown to have no effect on *in vitro* FANCD2 deubiquitination [55]. Upon USP1-UAF1 binding, the ID2_{Ub}-DNA complex is still in the closed-state conformation [55], whereby the Arm ID2 interface is formed around dsDNA by the FANCI and FANCD2 C termini [7,39,49,55] (Figs 2A, 5 and 6). However, the positions of FANCI and FANCD2 at the N-terminal ID2 interface (around FANCI's K523) are intermediate between the closed-state and the open-state ID2 conformation [55]. As a result, among all closed-state ID2 structures reported, the overall structure of USP1-UAF1-bound ID2_{Ub} complex is the most closely related to the nonubiquitinated open-state ID2 conformations. However, it is still substantially different from the intermediate-state ID2 structure reported upon FANCD2 ubiquitination [7] (Fig. 7).

Because FANCI ubiquitination is not predicted to occlude binding of UAF1 to FANCI (Fig. 6D), it has been suggested that USP1-UAF1 targets the ubiquitin of FANCD2 in dimonoubiquitinated ID2 complex (I_{Ub}-D2_{Ub}), in the same manner as in the ID2_{Ub} complex [55]. However, some subtle conformational changes upon FANCI ubiquitination may make this binding less effective. Indeed, pull-down experiments using a catalytically inactive GST-USP1-UAF1 as bait have revealed that USP1-UAF1 binds preferentially to ID2 complexes containing nonubiquitinated (rather than ubiquitinated) FANCI [27]. This is consistent with FANCD2 being deubiquitinated less efficiently in the I_{Ub}D2_{Ub}-DNA complex than in the ID2_{Ub}-DNA complex [20,30]. Further, biochemical experiments coupled with a USP1-UAF1-I_{Ub}D2_{Ub} structure are required to explain how FANCI's ubiquitination interferes with USP1-UAF1-catalyzed FANCD2 deubiquitination. More work is also required to answer how the N terminus of USP1 facilitates removal of ubiquitin from FANCD2, since no clear density for this USP1 segment was observed in the USP1-UAF1-ID2_{Ub}-DNA structure. Lastly, it is still not understood how interaction of ubiquitinated ID2 with DNA can influence USP1-UAF1-mediated deubiquitination. Despite the reported ability of USP1-UAF1 complex, or individual USP1/UAF1 proteins, to bind various DNA structures [31,65], no such interactions were observed in the USP1-UAF1-ID2_{Ub}-DNA structure [55] (Fig. 6A).

Molecular and cellular functions of FANCI/FANCD2 deubiquitination

The role of USP1-UAF1 might be more complicated than simply removing the ubiquitins in a timely

manner. At least one of the roles of USP1-UAF1 action (on ubiquitinated ID2 complexes) appears to be in enriching a dimonoubiquitinated ID2 (I_{Ub}D2_{Ub}) population [20,27,30]. This is achieved through USP1-UAF1 preferentially targeting the ID2_{Ub}-DNA complexes, which are more susceptible to cleavage than the more resistant I_{Ub}D2_{Ub}-DNA complexes [20,30]. Indeed, it has been shown that any imbalance in the ratio of ubiquitinated FANCD2 to ubiquitinated FANCI, caused by a faster rate of FANCD2 ubiquitination, can be corrected by USP1-UAF1 *in vitro*; FANCD2 has been shown to be deubiquitinated faster than FANCI, until an equilibrium can be reached whereby ubiquitinated FANCD2 and FANCI become equimolar [27]. USP1-UAF1 activity may also regulate the levels of ID2 in chromatin, by effectively reducing its affinity for dsDNA (since ID2 binds weaker to dsDNA than ID2_{Ub} and I_{Ub}D2_{Ub}). Indeed, loss of USP1 activity results in accumulation of FANCD2 on chromatin [21,22]. However, USP1-UAF1 may also act on ubiquitinated ID2 complex that has been removed from chromatin, since DNA-free dimonoubiquitinated ID2 complexes have been reported to be more susceptible to USP1-UAF1 activity [20,27]. The DVC1-p97 ubiquitin segregase complex has been proposed to remove from chromatin, ubiquitinated ID2 complexes that are additionally SUMOylated and subsequently polyubiquitinated, as a way of controlling ID2 dosage at DNA damage sites [66].

The biological role of USP1-UAF1 action on ID2 is less straightforward, since deleting or impairing expression of USP1 or UAF1 has consequences that are not restricted to ID2 ubiquitination. Apart from acting on monoubiquitinated FANCD2 and FANCI, the USP1-UAF1 complex is also known to remove ubiquitin from monoubiquitinated proliferating cell nuclear antigen (PCNA) [20,67], and recently, many more USP1-UAF1 targets have been discovered [68–77]. Unlike UAF1 deletion, which is embryonically lethal in mice [78], USP1 deletion is viable, but results in various defects, including increased perinatal lethality, male infertility, cross-linker hypersensitivity, and an FA-like phenotype [22]. At the cellular level, blocking USP1 activity leads to increased cytotoxicity in response to UV and cisplatin, as well as impaired homologous recombination and sister-chromatid exchange [79]. Many of these effects, though, may be attributed to functions of USP1-UAF1 on other proteins than ID2, and indeed, USP1 inhibition has been shown to affect both the FA pathway and DNA translesion synthesis, by inhibiting both FANCD2 and PCNA deubiquitination [79]. Hence, introduction of a USP1 mutant that is specifically defective against ubiquitinated FANCD2

PDB ID	6vad	6vaa	7kzq	7kzr	7kzs	7kzt	7kzv	6vaf	6vae	7ay1
6vad	0.0	0.5	3.1	1.5	1.5	1.9	2.5	2.1	2.0	1.4
6vaa	0.5	0.0	3.0	1.4	1.3	1.7	2.5	2.0	2.0	1.3
7kzq	3.1	3.0	0.0	1.0	1.0	1.4	2.5	2.3	2.2	2.2
7kzr	1.5	1.4	1.0	0.0	0.6	1.2	2.1	2.1	2.0	1.7
7kzs	1.5	1.3	1.0	0.6	0.0	1.2	2.1	2.1	2.0	1.8
7kzt	1.9	1.7	1.4	1.2	1.2	0.0	2.1	1.4	1.3	2.1
7kzv	2.5	2.5	2.5	2.1	2.1	2.1	0.0	2.6	2.6	2.2
6vaf	2.1	2.0	2.3	2.1	2.1	1.4	2.6	0.0	0.5	2.2
6vae	2.0	2.0	2.2	2.0	2.0	1.3	2.6	0.5	0.0	2.3
7ay1	1.4	1.3	2.2	1.7	1.8	2.1	2.2	2.2	2.3	0.0

FANCI

PDB ID	6vad	6vaa	7kzq	7kzr	7kzs	7kzt	7kzv	6vaf	6vae	7ay1
6vad	0.0	1.4	2.5	2.9	3.8	6.9	11.4	12.6	12.6	5.8
6vaa	1.4	0.0	2.2	3.2	3.9	10.4	11.1	12.5	12.6	5.9
7kzq	2.5	2.2	0.0	1.9	2.8	7.5	12.2	13.8	13.8	5.8
7kzr	2.9	3.2	1.9	0.0	3.5	7.0	11.9	14.2	14.1	5.4
7kzs	3.8	3.9	2.8	3.5	0.0	10.9	10.6	12.6	12.8	4.7
7kzt	6.9	10.4	7.5	7.0	10.9	0.0	9.2	11.6	11.6	7.3
7kzv	11.4	11.1	12.2	11.9	10.6	9.2	0.0	5.7	5.9	2.1
6vaf	12.6	12.5	13.8	14.2	12.6	11.6	5.7	0.0	0.6	2.8
6vae	12.6	12.6	13.8	14.1	12.8	11.6	5.9	0.6	0.0	2.9
7ay1	5.8	5.9	5.8	5.4	4.7	7.3	2.1	2.8	2.9	0.0

FANCD2

PDB ID	Complex	ID2 Conformation	RMSD values
6vad	ID2	Open	0.0
6vaa	ID2 - DNA		2.5
7kzq	FA core - ID2		5.0
7kzr	FA core - UBE2T - ID2		7.5
7kzs	FA core - UBE2T - ID2 - DNA		10.0
7kzt	FA core - UBE2T - ID2 - DNA	Intermediate	12.5
7kzv	FA core - UBE2T - ID2 - DNA	Closed	15.0
6vaf	ID2ub - DNA		
6vae	lubD2ub - DNA		
7ay1	USP1-UAF1 - ID2ub - DNA		

Fig. 7. Root-mean-square deviation (RMSD) values of FANCI and FANCD2 atomic positions in the ID2 complex between available PDB structures. Alpha carbon (C α) RMSD values for each protein were calculated using matchmaker tool in ChimeraX software for each PDB pair. PDB codes, corresponding complex and ID2 conformation (open, intermediate, or closed) are given. Higher RMSD values are highlighted with darker shade of red according to the table shown at bottom right.

(and/or FANCI), in cells, may be particularly useful for determining the biological role of USP1-UAF1 in the FA pathway. To this effect, deletion of targeted mutagenesis on USP1 N terminus has been shown to specifically impact on FANCD2 deubiquitination, without having much of an effect on FANCI or PCNA deubiquitination [20]. Similarly, a cellular UAF1 mutant that specifically blocks UAF1-FANCI interaction [55] (Fig. 6A–C) may be also useful in assessing USP1-UAF1's biological role in deubiquitinating FANCD2.

Despite any wide-reaching effects of USP1 depletion, USP1 activity seems to be specifically required for formation of FANCD2 damage foci. Indeed, a defect of both basal (S phase-specific) and DNA damage-induced FANCD2 foci has been observed upon USP1 deletion in mouse embryonic fibroblasts [22] and HeLa cells [80]. Moreover, treatment of U2OS cells with a USP1-specific inhibitor is also associated with a reduction in DNA damage-induced FANCD2 foci [79]. These effects are not due to impaired γ H2AX focus formation (which are required for formation of FANCD2 foci [81]), since γ H2AX focus formation was found to be unaffected by the loss of USP1 [22,80]. Moreover, USP1 deletion does not impair FANCD2 chromatin localization. In fact, loss of USP1 is associated with increased ubiquitination and enrichment of FANCD2 in chromatin [21,22]. Hence, USP1 action is likely required for correct localization of ID2 within the chromatin. One possibility of how this may occur is by USP1-UAF1 effectively limiting the population of ID2 that is ubiquitinated. This may be necessary to restrict the $I_{Ub}D2_{Ub}$ population to sites of DNA damage or replication arrest (where ubiquitination occurred), as opposed to a widespread distribution across the chromatin. Clearly, more work is required to determine whether USP1-UAF1 action contributes to the formation or maintenance of ID2 foci, and how this achieved.

Perspective

Our knowledge of the requirements and mechanism of site-specific FANCD2 ubiquitination and deubiquitination has greatly expanded over the last few years. Very recently, structural and biochemical studies have also revealed that ubiquitin conjugation on FANCD2 facilitates clamping of ID2 complex on DNA. Furthermore, cryo-EM structures have provided insights into how the FA-core complex engages ID2 for FANCD2 ubiquitination, and how USP1-UAF1 engages the $ID2_{Ub}$ complex for ubiquitin removal. What is less clear is how the FA-core complex targets the ID2

complex for FANCI ubiquitination, and how this ubiquitin is subsequently removed by USP1-UAF1. FANCI ubiquitination has been found to largely protect the ubiquitin of FANCD2 from USP1-UAF1 activity. However, this may not be the only function of FANCI ubiquitination. That is because an $ID2$ complex where only FANCI is ubiquitinated ($I_{Ub}D2$) may be formed upon FANCI/ $ID2$ ubiquitination and/or $I_{Ub}D2_{Ub}$ deubiquitination. Our knowledge on such a complex is only that it also has a higher affinity for dsDNA than the $ID2$ complex, albeit not as high as that of $ID2_{Ub}/I_{Ub}D2_{Ub}$ complexes [30]. What is also less clear is the mechanism by which DNA can influence $ID2$ deubiquitination. Lastly, despite the fact that (at least part of) the molecular functions of FANCD2/FANCI ubiquitination have now been uncovered, it is still poorly understood how FANCD2/FANCI ubiquitination and deubiquitination actually contribute to $ID2$ foci, ICL repair, and maintenance of genomic stability. Clearly, more work is required to answer these questions and improve further our understanding of the mechanisms and roles of $ID2$ ubiquitination and deubiquitination. Instrumental for these are expected to be the recent advances in preparation of ubiquitinated FANCI and FANCD2, coupled with the ability to now obtain high-resolution cryo-EM structures of protein complexes.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KL wrote the original draft with help from CA. MR calculated FANCI and FANCD2 $C\alpha$ RMSD values. KL made the figures. KL, CA, MR, and HW reviewed and accepted the final manuscript.

References

- 1 Nalepa G & Clapp DW (2018) Fanconi anaemia and cancer: an intricate relationship. *Nat Rev Cancer* **18**, 168–185.
- 2 Niraj J, Färkkilä A & D'Andrea AD (2019) The Fanconi anemia pathway in cancer. *Annu Rev Cancer Biol* **3**, 457–478.

- 3 Shen X, Wang R, Kim MJ, Hu Q, Hsu CC, Yao J, Klages-Mundt N, Tian Y, Lynn E, Brewer TF *et al.* (2020) A surge of DNA damage links transcriptional reprogramming and hematopoietic deficit in Fanconi anemia. *Mol Cell* **80**, 1013–1024.e6.
- 4 Dingler FA, Wang M, Mu A, Millington CL, Oberbeck N, Watcham S, Pontel LB, Kamimae-Lanning AN, Langevin F, Nadler C *et al.* (2020) Two aldehyde clearance systems are essential to prevent lethal formaldehyde accumulation in mice and humans. *Mol Cell* **80**, 996–1012.e9.
- 5 Renaudin X & Rosselli F (2020) The FANCI/BRCA pathway releases replication blockades by eliminating DNA interstrand cross-links. *Genes (Basel)* **11**, 585.
- 6 Shakeel S, Rajendra E, Alcón P, O'Reilly F, Chorev DS, Maslen S, Degliesposti G, Russo CJ, He S, Hill CH *et al.* (2019) Structure of the Fanconi anemia monoubiquitin ligase complex. *Nature* **575**, 234–237.
- 7 Wang S, Wang R, Peralta C, Yaseen A & Pavletich NP (2021) Structure of the FA core ubiquitin ligase closing the ID clamp on DNA. *Nat Struct Mol Biol* **28**, 300–309.
- 8 Rajendra E, Oestergaard VH, Langevin F, Wang M, Dornan GL, Patel KJ & Passmore LA (2014) The genetic and biochemical basis of FANCD2 monoubiquitination. *Mol Cell* **54**, 858–869.
- 9 Meetei AR, de Winter JP, Medhurst AL, Wallisch M, Waisfisz Q, van de Vrugt HJ, Oostra AB, Yan Z, Ling C, Bishop CE *et al.* (2003) A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat Genet* **35**, 165–170.
- 10 Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D'Andrea AD & Dutta A (2006) UBE2T Is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol Cell* **23**, 589–596.
- 11 Hodson C, Purkiss A, Miles JA & Walden H (2014) Structure of the human FANCL RING-Ube2T complex reveals determinants of cognate E3–E2 selection. *Structure* **22**, 337–344.
- 12 Chaugule VK, Arkinson C, Rennie ML, Kämäräinen O, Toth R & Walden H (2020) Allosteric mechanism for site-specific ubiquitination of FANCD2. *Nat Chem Biol* **16**, 291–301.
- 13 Taniguchi T, Garcia-Higuera I, Andreassen PR, Gregory RC, Grompe M & D'Andrea AD (2002) S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood* **100**, 2414–2420.
- 14 Smogorzewska A, Matsuoka S, Vinciguerra P, McDonald ER, Hurov KE, Luo J, Ballif BA, Gygi SP, Hofmann K, D'Andrea AD *et al.* (2007) Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* **129**, 289–301.
- 15 Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M & D'Andrea AD (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell* **7**, 249–262.
- 16 Sims AE, Spiteri E, Sims RJ, Arita AG, Lach FP, Landers T, Wurm M, Freund M, Neveling K, Hanenberg H *et al.* (2007) FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nat Struct Mol Biol* **14**, 564–567.
- 17 Howlett NG, Taniguchi T, Durkin SG, D'Andrea AD & Glover TW (2005) The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. *Hum Mol Genet* **14**, 693–701.
- 18 Nijman SMB, Huang TT, Dirac AMG, Brummelkamp TR, Kerkhoven RM, D'Andrea AD & Bernards R (2005) The deubiquitinating enzyme USP1 regulates the fanconi anemia pathway. *Mol Cell* **17**, 331–339.
- 19 Cohn MA, Kowal P, Yang K, Haas W, Huang TT, Gygi SP & D'Andrea AD (2007) A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. *Mol Cell* **28**, 786–797.
- 20 Arkinson C, Chaugule VK, Toth R & Walden H (2018) Specificity for deubiquitination of monoubiquitinated FANCD2 is driven by the N-terminus of USP1. *Life Sci Alliance* **1**, e201800162.
- 21 Oestergaard VH, Langevin F, Kuiken HJ, Pace P, Niedzwiedz W, Simpson LJ, Ohzeki M, Takata M, Sale JE & Patel KJ (2007) Deubiquitination of FANCD2 is required for DNA crosslink repair. *Mol Cell* **28**, 798–809.
- 22 Kim JM, Parmar K, Huang M, Weinstock DM, Ruit CA, Kutok JL & D'Andrea AD (2009) Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. *Dev Cell* **16**, 314–320.
- 23 Murai J, Yang K, Dejsuphong D, Hirota K, Takeda S & D'Andrea AD (2011) The USP1/UAF1 complex promotes double-strand break repair through homologous recombination. *Mol Cell Biol* **31**, 2462–2469.
- 24 Joo W, Xu G, Persky NS, Smogorzewska A, Rudge DG, Buzovetsky O, Elledge SJ & Pavletich NP (2011) Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. *Science* **333**, 312–316.
- 25 Sato K, Toda K, Ishiai M, Takata M & Kurumizaka H (2012) DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. *Nucleic Acids Res* **40**, 4553–4561.
- 26 Longerich S, Kwon Y, Tsai MS, Hlaing AS, Kupfer GM & Sung P (2014) Regulation of FANCD2 and FANCI monoubiquitination by their interaction and by DNA. *Nucleic Acids Res* **42**, 5657–5670.
- 27 van Twest S, Murphy VJ, Hodson C, Tan W, Swuec P, O'Rourke JJ, Heierhorst J, Crismani W & Deans AJ (2017) Mechanism of ubiquitination and deubiquitination in the Fanconi anemia pathway. *Mol Cell* **65**, 247–259.
- 28 Liang Z, Liang F, Teng Y, Chen X, Liu J, Longerich S, Rao T, Green AM, Collins NB, Xiong Y *et al.* (2019)

- Binding of FANCI-FANCD2 complex to RNA and R-loops stimulates robust FANCD2 monoubiquitination. *Cell Rep* **26**, 564–572.e5.
- 29 Liang CC, Li Z, Lopez-Martinez D, Nicholson WV, Vénien-Bryan C & Cohn MA (2016) The FANCD2-FANCI complex is recruited to DNA interstrand crosslinks before monoubiquitination of FANCD2. *Nat Commun* **7**, 12124.
 - 30 Rennie ML, Lemonidis K, Arkinson C, Chaugule VK, Clarke M, Streetley J, Spagnolo L & Walden H (2020) Differential functions of FANCI and FANCD2 ubiquitination stabilize ID2 complex on DNA. *EMBO Rep* **21**, e50133.
 - 31 Liang F, Miller AS, Longerich S, Tang C, Maranon D, Williamson EA, Hromas R, Wiese C, Kupfer GM & Sung P (2019) DNA requirement in FANCD2 deubiquitination by USP1-UAF1-RAD51AP1 in the Fanconi anemia DNA damage response. *Nat Commun* **10**, 2849.
 - 32 Andreassen PR, D'Andrea AD & Taniguchi T (2004) ATR couples FANCD2 monoubiquitination to the DNA-damage response. *Genes Dev* **18**, 1958–1963.
 - 33 Ishiai M, Kitao H, Smogorzewska A, Tomida J, Kinomura A, Uchida E, Saberi A, Kinoshita E, Kinoshita-Kikuta E, Koike T *et al.* (2008) FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. *Nat Struct Mol Biol* **15**, 1138–1146.
 - 34 Chen YH, Jones MJK, Yin Y, Crist SB, Colnaghi L, Sims RJ, Rothenberg E, Jallepalli PV & Huang TT (2015) ATR-mediated phosphorylation of FANCI regulates dormant origin firing in response to replication stress. *Mol Cell* **58**, 323–338.
 - 35 Cheung RS, Castella M, Abeyta A, Gafken PR, Tucker N & Taniguchi T (2017) Ubiquitination-linked phosphorylation of the FANCI S/TQ cluster contributes to activation of the Fanconi anemia I/D2 complex. *Cell Rep* **19**, 2432–2440.
 - 36 Tan W, van Twest S, Murphy VJ & Deans AJ (2020) ATR-mediated FANCI phosphorylation regulates both ubiquitination and deubiquitination of FANCD2. *Front Cell Dev Biol* **8**, 1–11.
 - 37 Lopez-Martinez D, Kupculak M, Yang D, Yoshikawa Y, Liang C-C, Wu R, Gygi SP & Cohn MA (2019) Phosphorylation of FANCD2 inhibits the FANCD2/FANCI complex and suppresses the Fanconi anemia pathway in the absence of DNA damage. *Cell Rep* **27**, 2990–3005.e5.
 - 38 Yuan F, El Hokayem J, Zhou W & Zhang Y (2009) FANCI protein binds to DNA and interacts with FANCD2 to recognize branched structures. *J Biol Chem* **284**, 24443–24452.
 - 39 Wang R, Wang S, Dhar A, Peralta C & Nikola P (2020) DNA clamp function of the mono - ubiquitinated Fanconi anemia FANCI - FANCD2 complex. *Nature* **580**, 278–282.
 - 40 Alpi AF, Pace PE, Babu MM & Patel KJ (2008) Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol Cell* **32**, 767–777.
 - 41 Jeong E, Lee S-G, Kim H-S, Yang J, Shin J, Kim Y, Kim J, Schärer OD, Kim Y, Yeo J-E *et al.* (2020) Structural basis of the Fanconi anemia-associated mutations by the FANCA and FANCG complex. *Nucleic Acids Res* **48**, 3328–3342.
 - 42 Swuec P, Renault L, Borg A, Shah F, Murphy VJ, van Twest S, Snijders AP, Deans AJ & Costa A (2017) The FA core complex contains a homo-dimeric catalytic module for the symmetric mono-ubiquitination of FANCI-FANCD2. *Cell Rep* **18**, 611–623.
 - 43 Huang Y, Leung JWC, Lowery M, Matsushita N, Wang Y, Shen X, Huong D, Takata M, Chen J & Li L (2014) Modularized functions of the Fanconi anemia core complex. *Cell Rep* **7**, 1849–1857.
 - 44 Pace P, Johnson M, Tan WM, Mosedale G, Sng C, Hoatlin M, De Winter J, Joenje H, Gergely F & Patel KJ (2002) Fance: the link between Fanconi anaemia complex assembly and activity. *EMBO J* **21**, 3414–3423.
 - 45 Nookala RK, Hussain S & Pellegrini L (2007) Insights into Fanconi anaemia from the structure of human FANCE. *Nucleic Acids Res* **35**, 1638–1648.
 - 46 Cole AR, Lewis LPC & Walden H (2010) The structure of the catalytic subunit FANCL of the Fanconi anemia core complex. *Nat Struct Mol Biol* **17**, 294–298.
 - 47 Hodson C, Cole AR, Lewis LPC, Miles JA, Purkiss A & Walden H (2011) Structural analysis of human FANCL, the E3 ligase in the Fanconi anemia pathway. *J Biol Chem* **286**, 32628–32637.
 - 48 Miles JA, Frost MG, Carroll E, Rowe ML, Howard MJ, Sidhu A, Chaugule VK, Alpi AF & Walden H (2015) The Fanconi anemia DNA repair pathway is regulated by an interaction between ubiquitin and the E2-like fold domain of FANCL. *J Biol Chem* **290**, 20995–21006.
 - 49 Alcón P, Shakeel S, Chen ZA, Rappsilber J, Patel KJ & Passmore LA (2020) FANCD2–FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. *Nat Struct Mol Biol* **27**, 240–248.
 - 50 Beck M, Schmidt A, Malmstroem J, Claassen M, Ori A, Szymborska A, Herzog F, Rinner O, Ellenberg J & Aebersold R (2011) The quantitative proteome of a human cell line. *Mol Syst Biol* **7**, 549.
 - 51 Sondalle SB, Longerich S, Ogawa LM, Sung P & Baserga SJ (2019) Fanconi anemia protein FANCI functions in ribosome biogenesis. *Proc Natl Acad Sci USA* **117**, 2561–2570.
 - 52 Dikic I, Wakatsuki S & Walters KJ (2009) Ubiquitin-binding domains from structures to functions. *Nat Rev Mol Cell Biol* **10**, 659–671.

- 53 Tan W, van Twest S, Leis A, Bythell-Douglas R, Murphy VJ, Sharp M, Parker MW, Crismani WM & Deans AJ (2020) Monoubiquitination by the human Fanconi anemia core complex clamps FANCI: FANCD2 on DNA in filamentous arrays. *eLife* **9**, e54128.
- 54 Schlacher K, Wu H & Jasin M (2012) A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* **22**, 106–116.
- 55 Rennie ML, Arkinson C, Chaugule VK, Toth R & Walden H (2021) Structural basis of FANCD2 deubiquitination by USP1–UAF1. *Nat Struct Mol Biol* **28**, 356–364.
- 56 Komander D, Clague MJ & Urbé S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550–563.
- 57 Cohn MA, Kee Y, Haas W, Gygi SP & D'Andrea AD (2009) UAF1 is a subunit of multiple deubiquitinating enzyme complexes. *J Biol Chem* **284**, 5343–5351.
- 58 Li H, Lim KS, Kim H, Hinds TR, Jo U, Mao H, Weller CE, Sun J, Chatterjee C, D'Andrea AD *et al.* (2016) Allosteric activation of ubiquitin-specific proteases by β -propeller proteins UAF1 and WDR20. *Mol Cell* **63**, 249–260.
- 59 Dharadhar S, Clerici M, van Dijk WJ, Fish A & Sixma TK (2016) A conserved two-step binding for the UAF1 regulator to the USP12 deubiquitinating enzyme. *J Struct Biol* **196**, 437–447.
- 60 Yin J, Schoeffler AJ, Wickliffe K, Newton K, Starovasnik MA, Dueber EC & Harris SF (2015) Structural insights into WD-repeat 48 activation of ubiquitin-specific protease 46. *Structure* **23**, 2043–2054.
- 61 Kee Y, Yang K, Cohn MA, Haas W, Gygi SP & D'Andrea AD (2010) WDR20 regulates activity of the USP12-UAF1 deubiquitinating enzyme complex. *J Biol Chem* **285**, 11252–11257.
- 62 Dahlberg CL & Joo P (2014) The WD40-repeat proteins WDR-20 and WDR-48 bind and activate the deubiquitinating enzyme USP-46 to promote the abundance of the glutamate receptor GLR-1 in the ventral nerve cord of *Caenorhabditis elegans*. *J Biol Chem* **289**, 3444–3456.
- 63 Dharadhar S, Dijk WJ, Scheffers S, Fish A & Sixma TK (2021) Insert L1 is a central hub for allosteric regulation of USP1 activity. *EMBO Rep* **22**, e51749.
- 64 Yang K, Moldovan GL, Vinciguerra P, Murai J, Takeda S & D'Andrea AD (2011) Regulation of the Fanconi anemia pathway by a SUMO-like delivery network. *Genes Dev* **25**, 1847–1858.
- 65 Lim KS, Li H, Roberts EA, Gaudiano EF, Clairmont C, Sambel LA, Ponnieselvan K, Liu JC, Yang C, Kozono D *et al.* (2018) USP1 is required for replication fork protection in BRCA1-deficient tumors. *Mol Cell* **72**, 925–941.e4.
- 66 Gibbs-Seymour I, Oka Y, Rajendra E, Weinert BT, Passmore LA, Patel KJ, Olsen JV, Choudhary C, Bekker-Jensen S & Mailand N (2015) Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol Cell* **57**, 150–164.
- 67 Huang TT, Nijman SMB, Mirchandani KD, Galardy PJ, Cohn MA, Haas W, Gygi SP, Ploegh HL, Bernards R & D'Andrea AD (2006) Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat Cell Biol* **8**, 339–347.
- 68 Yu Z, Song H, Jia M, Zhang J, Wang W, Li Q, Zhang L & Zhao W (2017) USP1-UAF1 deubiquitinase complex stabilizes TBK1 and enhances antiviral responses. *J Exp Med* **214**, 3553–3563.
- 69 Sonogo M, Pellarin I, Costa A, Vinciguerra GLR, Coan M, Kraut A, D'Andrea S, Dall'Acqua A, Castillo-Tong DC, Califano D *et al.* (2019) USP1 links platinum resistance to cancer cell dissemination by regulating Snail stability. *Sci Adv* **5**, eaav3235.
- 70 Ma L, Lin K, Chang G, Chen Y, Yue C, Guo Q, Zhang S, Jia Z, Huang TT, Zhou A *et al.* (2019) Aberrant activation of b-catenin signaling drives glioma tumorigenesis via USP1-mediated stabilization of EZH2. *Cancer Res* **79**, 72–85.
- 71 Cui SZ, Lei ZY, Guan TP, Fan LL, Li YQ, Geng XY, Fu DX, Jiang HW & Xu SH (2020) Targeting USP1-dependent KDM4A protein stability as a potential prostate cancer therapy. *Cancer Sci* **111**, 1567–1581.
- 72 Goldbraikh D, Neufeld D, Eid-Mutlak Y, Lasry I, Gilda JE, Parnis A & Cohen S (2020) USP1 deubiquitinates Akt to inhibit PI3K-Akt-FoxO signaling in muscle during prolonged starvation. *EMBO Rep* **21**, e48791.
- 73 Li Y, Xu Y, Gao C, Sun Y, Zhou K, Wang P, Cheng J, Guo W, Ya C, Fan J *et al.* (2020) USP1 maintains the survival of liver circulating tumor cells by deubiquitinating and stabilizing TBLR1. *Front Oncol* **10**, 2062.
- 74 Niu Z, Li X, Feng S, Huang Q, Zhuang T, Yan C, Qian H, Ding Y, Zhu J & Xu W (2020) The deubiquitinating enzyme USP1 modulates ER α and modulates breast cancer progression. *J Cancer* **11**, 6992–7000.
- 75 Mussell A, Shen H, Chen Y, Mastro M, Eng KH, Bshara W, Frangou C & Zhang J (2020) USP1 regulates TAZ protein stability through ubiquitin modifications in breast cancer. *Cancers (Basel)* **12**, 3090.
- 76 Song H, Zhao C, Yu Z, Li Q, Yan R, Qin Y, Jia M & Zhao W (2020) UAF1 deubiquitinase complexes facilitate NLRP3 inflammasome activation by promoting NLRP3 expression. *Nat Commun* **11**, 1–11.
- 77 Han D, Wang L, Chen B, Zhao W, Liang Y, Li Y, Zhang H, Liu Y, Wang X, Chen T *et al.* (2021) USP1-WDR48 deubiquitinase complex enhances TGF- β

- induced epithelial–mesenchymal transition of TNBC cells via stabilizing TAK1. *Cell Cycle* **20**, 320–331.
- 78 Park E, Kim JM, Primack B, Weinstock DM, Moreau LA, Parmar K & D’Andrea AD (2013) Inactivation of Uaf1 causes defective homologous recombination and early embryonic lethality in mice. *Mol Cell Biol* **33**, 4360–4370.
- 79 Liang Q, Dexheimer TS, Zhang P, Rosenthal AS, Villamil MA, You C, Zhang Q, Chen J, Ott CA, Sun H *et al.* (2014) A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. *Nat Chem Biol* **10**, 298–304.
- 80 Guervilly JH, Renaud E, Takata M & Rosselli F (2011) USP1 deubiquitinase maintains phosphorylated CHK1 by limiting its DDB1-dependent degradation. *Hum Mol Genet* **20**, 2171–2181.
- 81 Bogliolo M, Lyakhovich A, Callén E, Castellà M, Cappelli E, Ramírez MJ, Creus A, Marcos R, Kalb R, Neveling K *et al.* (2007) Histone H2AX and Fanconi anemia FANCD2 function in the same pathway to maintain chromosome stability. *EMBO J* **26**, 1340–1351.
- 82 Sheng Y, Hong JH, Doherty R, Srikumar T, Shloush J, Avvakumov GV, Walker JR, Xue S, Neculai D, Wan JW *et al.* (2012) A human ubiquitin conjugating enzyme (E2)-HECT E3 ligase structure-function screen. *Mol Cell Proteomics* **11**, 329–341.