

Review Article

# Mechanism and disease association of E2-conjugating enzymes: lessons from UBE2T and UBE2L3

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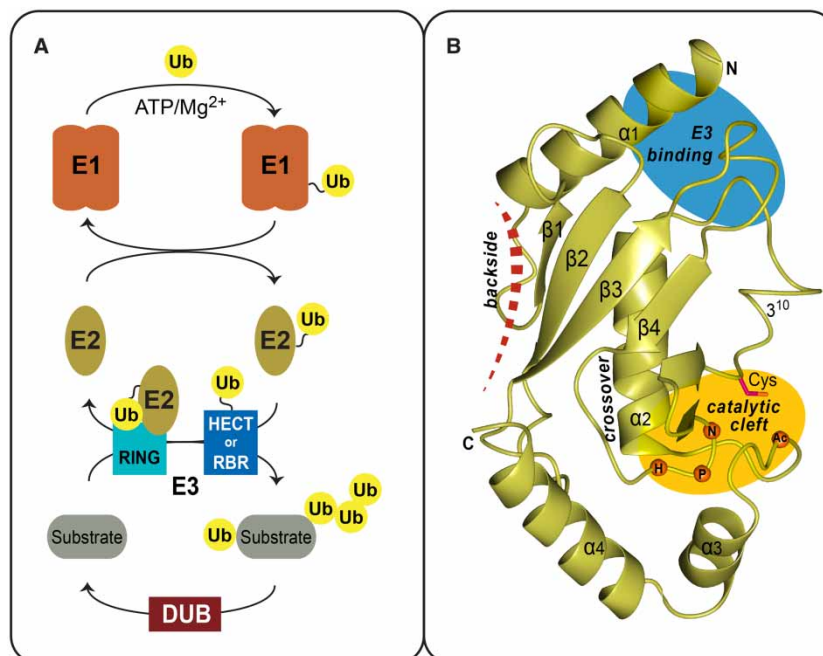
Ubiquitin signalling is a fundamental eukaryotic regulatory system, controlling diverse cellular functions. A cascade of E1, E2, and E3 enzymes is required for assembly of distinct signals, whereas an array of deubiquitinases and ubiquitin-binding modules edit, remove, and translate the signals. In the centre of this cascade sits the E2-conjugating enzyme, relaying activated ubiquitin from the E1 activating enzyme to the substrate, usually via an E3 ubiquitin ligase. Many disease states are associated with dysfunction of ubiquitin signalling, with the E3s being a particular focus. However, recent evidence demonstrates that mutations or impairment of the E2s can lead to severe disease states, including chromosome instability syndromes, cancer predisposition, and immunological disorders. Given their relevance to diseases, E2s may represent an important class of therapeutic targets. In the present study, we review the current understanding of the mechanism of this important family of enzymes, and the role of selected E2s in disease.

## Introduction

Protein ubiquitination is a reversible post-translational modification that modulates a variety of homeostatic processes in eukaryotic cells. The modification typically involves the sequential action of activating enzymes (E1s), conjugating enzymes (E2s), and ligase enzymes (E3s), resulting in an isopeptide link between the C-terminus of ubiquitin (Ub) and a specific lysine on a target protein [1]. Briefly, E1s activate the C-terminus of ubiquitin in an ATP/Mg<sup>2+</sup>-dependent manner and load ubiquitin onto a conserved catalytic cysteine within E2s (denoted as E2~Ub). Subsequently, hundreds of different E3s can then associate with one or more E2~Ub to catalyze the ubiquitination of substrate targets (Figure 1A). The surface of ubiquitin itself presents several conserved functional patches, for example, the Ile44-hydrophobic patch (Leu8/Ile44/His68/Val70), which sustains crucial non-covalent interactions during ubiquitination as well as in signalling [2,3]. In addition, any of the seven surface lysines, or the N-terminus of ubiquitin, supports further building of ubiquitin chains that adopt distinct topologies [4]. Ubiquitin-binding domains are present within numerous proteins and can discriminate the different ubiquitin chain types, thereby facilitating signal cascades and protein networks [5]. Post-modification, enzymes called deubiquitinating enzymes (DUBs) can alter or erase the ubiquitin signals, thus modulating the event [6] (Figure 1A). Ubiquitin-like proteins (Ubls), such as SUMO (small ubiquitin-like modifier) and NEDD8 (neural precursor cell expressed developmentally down-regulated protein 8), also take part in post-translation modifications. These have a similar, but smaller, cohort of proteins that regulate the modification and have been reviewed elsewhere [7,8]. An intricate balance between E3 ligase and DUB activities regulates the specificity of the ubiquitin signal. Unsurprisingly, deregulation of these enzymes has been linked to several cancers and neurodegenerative diseases [9–12]. In addition to the large E3 and DUB classes, recent studies illustrate how E2s are

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**Figure 1. Ubiquitin pathway and the E2 fold.**

(A) Overview of the Ub pathway and the enzymes involved at each step: activation (E1), conjugation (E2), ligation (E3), and deubiquitination (DUB). The E1 mediates ubiquitin activation in an energy-consuming step. The ubiquitin thioester is then transferred onto a catalytic cysteine of the E2 enzyme. RING-type E3s form a non-covalent complex with the E2~Ub thioester intermediate or, alternatively, ubiquitin is transferred to catalytic sites of HECT and RBR-type E3 ligases. The E3 enzymes ultimately catalyze ubiquitination of a substrate lysine. Ubiquitin signals can also be extended to form polyubiquitin chains. Finally, DUBs catalyze the removal of ubiquitin. (B) Ribbon diagram of UBE2D2 (PDB 2ESK) as a representative of the UBC fold conserved among ubiquitin E2s. Secondary structure elements and the termini of the domain are indicated. Also depicted is the location of the active-site cysteine within the catalytic cleft (yellow oval), the E3-binding region (blue oval), the cross-over helix that stabilizes ‘closed’ conformers of the E2~Ub thioester intermediate and location of the E2 backside-binding surface (red dashed line).

more than just intermediaries of the ubiquitin pathway, but have multifaceted roles in human physiology and pathology. What follows is a review of our current mechanistic understanding of E2s, and the role of selected E2s in disease.

### Structure–function overview of the E2 fold

Humans have around 35 ubiquitin E2 family members, all sharing a core ubiquitin conjugation (UBC) domain that spans roughly 150 residues. Previously, E2s were classified by their UBC domain extensions; class I E2s have only the core domain, classes II and III have N- or C-terminal extensions respectively, and class IV are extended at both ends [13]. The extensions in the majority of classes II and III E2s are largely conserved, but lack secondary structure; they influence localization, E1/E3 interactions, or the ubiquitination event. Class IV enzymes, such as UBE2O and BIRC6, are significantly larger (>1200 residues) and are likely to be multidomain proteins. Currently, the *HUGO Gene Nomenclature Committee* has formally categorized mammalian E2s using the syntax UBE2X<sub>n</sub>, where X and n denote a letter and number, respectively (<http://www.genenames.org/cgi-bin/genefamilies/set/102>). UBC folds (Figure 1B) contain an N-terminal helix ( $\alpha$ 1), a four-stranded  $\beta$ -meander ( $\beta$ 1–4), a short  $3_{10}$ -helix that leads into the central ‘cross-over’ helix ( $\alpha$ 2), and two C-terminal helices ( $\alpha$ 3 and 4) [14,15]. The E2 catalytic cleft is located in a shallow groove with the active-site cysteine preceding the  $3_{10}$ -helix. The catalytic cysteine is structurally supported by a tri-peptide His-Pro-Asn motif that is a conserved UBC fold feature [16,17]. Certain ubiquitin E2s vary slightly in the tri-peptide sequence, for example His-Pro-His in UBE2W, whereas others like the UBE2J and UBE2Q family completely lack the motif.

Nevertheless, the variations do not abolish the ubiquitin conjugation activity in these E2s [18]. In contrast, the UBE2V family is devoid of both the catalytic cysteine and the His-Pro-Asn motif, and these proteins function as cofactors for the UBE2N enzyme. Also present in the catalytic cleft is a crucial, negatively charged, residue (typically Asp) in the  $\alpha 3$ – $\alpha 4$  loop that aligns the incoming substrate lysine towards the E2 catalytic cysteine [19]. Alternatively, in certain E2s, for example UBE2A, a neutral serine is present at the analogous position and phosphorylation of this residue can regulate the E2 enzyme function [20–23].

Ubiquitin can be activated by two distinct E1s: UBA1 and UBA6, with UBA1 capable of loading ubiquitin on the majority of E2s [24–26]. Structure–function studies of ubiquitin and Ubl E1s have uncovered details of the activation process and are reviewed elsewhere [27,28]. Interestingly, in mammalian cell lines, certain E2s (for example, the UBE2R family) are constitutively loaded with ubiquitin [25]. Structures of E2~Ub thioester mimics reflect the dynamic conformations adopted by ubiquitin relative to the E2 [29–31]. In particular, the UBE2N~Ub mimic resembles a compact ‘closed’ conformation more frequently than does a UBE2D3~Ub mimic. The different conformers can be indicative of the ubiquitination potential of the E2. A closed E2~Ub conformation is stabilized by interactions between the Ub Ile44-hydrophobic patch and conserved hydrophobic residues on the E2 cross-over helix. In UBE2S, UBE2R1, and UBE2G2, this closed conformation promotes efficient ubiquitin discharge, as well as E3-independent ubiquitin chain formation [32,33]. In addition, some E2s are capable of building linkage-specific polyubiquitin chains that involve additional interactions between the E2 and the acceptor ubiquitin surface surrounding the target lysine. In case of UBE2K [34,35], residues around the UBC catalytic cleft position the Lys48 surface of the acceptor ubiquitin, whereas C-terminal helices of UBE2S [33,36] orient the Lys-11 surface of the incoming ubiquitin. In contrast, the Lys63-specific UBE2N relies on an inactive E2 partner UBE2V1/2 to orientate the acceptor ubiquitin surface [37]. In the case of UBE2G2, homodimerization of E2~Ub intermediate facilitates both donor and acceptor ubiquitin surfaces required for catalysis and results in assembly of Lys48-linked ubiquitin chains on E2 catalytic cysteine [38–40]. Other Lys48-specific E2s, including the UBE2G and UBE2R families [41–43], have insertions of variable lengths near the catalytic cleft. These facilitate linkage-specific products via different modes and have been reviewed elsewhere [44]. In the majority of cases, linkage-specific E2s extend previously monoubiquitinated substrates. This enables substrate polyubiquitination to be delineated into distinct events, that is ubiquitin chain initiation followed by elongation [45–47]. Initial ubiquitin conjugation, or lysine-specific monoubiquitination on substrates, almost always requires an E3. A contrasting example is the UBE2E family where disordered N-terminal extensions impede polyubiquitination events [48]. Remarkably, this E2 family mediates an E3-independent, site-specific monoubiquitination of the DNA methyltransferase SETDB1 and, consequently, stimulates the methyltransferase activity [49]. Furthermore, direct cross-talk between the DUB enzyme OTUB1 and several E2s, including the UBE2E family, is observed during DNA damage-associated ubiquitination [50]. Structural analyses reveal how ubiquitin from an E2~Ub intermediate occupies the proximal ubiquitin-binding site of OTUB1, while free ubiquitin binds the distal site. The ternary E2~Ub/OTUB1/Ub complex inhibits both ubiquitin discharge from the E2~Ub intermediate and the DUB activity of OTUB1 [51,52].

Towards the N-terminus of the UBC fold are partially overlapping surfaces ( $\alpha 1$ , the  $\beta 2$ – $\beta 3$  loop, and the  $3_{10}$ -to- $\alpha 2$  loop) involved in interactions with both the E1 and E3 (Figure 1B). This overlap ensures that the E1–E2- and E2–E3-binding events are mutually exclusive during ubiquitin transfer, thus regulating the flow of the pathway [53,54]. Interactions of E2–E3 are usually weak and transient in nature, with dissociation constants in the high micromolar range. Conserved E2 residues that support the canonical E2–E3 interaction include polar residues in the N-terminal helix (typically Arg/Lys) and hydrophobic residues in the  $\beta 2$ – $\beta 3$  loop (typically Phe) and the  $3_{10}$ -to- $\alpha 2$  loop (typically Pro and Ala). Structures of the UBE2D family in complex with different E3s reveal how the E2 surface offers plasticity in the E2–E3 interaction [55–57]. Interestingly, E3 binding does not induce major conformational changes within the E2 UBC fold. Given that the E3-binding site is remote from the E2 catalytic cleft, the underlying mechanism forming a productive E2–E3 complex was not immediately clear. E3 ligases are broadly classified by how they catalyze the ubiquitination event. Briefly, really interesting new gene (RING)/U-box E3s (reviewed in ref. [58]) mediate direct transfer of ubiquitin from E2~Ub to the target substrate, whereas the homologous to the E6-AP C-terminus (HECT) and RBR E3s (reviewed in refs [59,60]) form an additional ubiquitin thioester intermediate on a conserved catalytic cysteine (E3~Ub) prior to substrate modification (Figure 1A). This distinction results in RING E3s being dependent on the partner E2 for regulating the ubiquitin product type, whereas HECT and RBR E3s can potentially overrule any linkage-specific bias. The different modes of ubiquitin transfer suggested that a productive E2~Ub–E3 complex might differ depending on the type of E3 involved. Recent structural and biochemical analyses of the UBE2D family have

revealed that HECT/RBR E3s do not require a closed E2~Ub conformation to catalyze ubiquitination [61–64]. In contrast, RING/U-box E3s promote a closed E2~Ub conformation, and mutations in the E2 cross-over helix that destabilize this result in the loss of ubiquitination [19,65,66]. Structures of an E2~Ub mimic in complex with RING/U-box E3s also reveal critical contacts between E3s and ubiquitin that influence ubiquitination. First, a conserved E3 residue, typically Arg, makes connections with the Ile<sup>36</sup> hydrophobic patch of ubiquitin as well as with the E2 main chain. These connections are essential for all RING/U-box-mediated ubiquitination; consequently, this residue has been denoted the allosteric ‘linchpin’. Second, the ubiquitin is further buttressed by contacts with homo-/hetero-dimeric partners of the RING domains, or by non-RING elements [19,65,66]. Taken together, these studies demonstrate how RING domain E3s, previously considered as mere molecular scaffolds, can actively influence the E2~Ub intermediate for ubiquitination. Substrate binding and specificity, fundamentally an E3 attribute, can also involve direct E2–substrate interactions, as seen with the PCGF4 (polycomb group RING finger)/RING1B–UBE2D3 complex bound to the nucleosome target [67]. However, how the E2~Ub/E3 modules interact with their non-ubiquitin-based substrates is largely unexplored.

An interaction surface of increasing interest is the ‘E2 backside’ located on the  $\beta$ -meander, distal to the catalytic cleft (Figure 1B). In the UBE2D family, this surface supports non-covalent ubiquitin interactions that stimulate processivity of the E2 enzyme [68,69]. Several RING E3s also harbour ancillary E2 backside-binding regions that induce different allosteric effects, such as stabilization of the canonical RING–E2 interface, E2~Ub charging, and processive ubiquitination [70–74]. Interestingly, in certain E2s, the backside interaction with E3s can regulate the type of ubiquitin signal generated on substrates [75,76]. Currently, functional roles for the E2 backside surface are observed in a quarter of all ubiquitin E2s and are predominantly coupled with RING E3s. Given the versatility of this surface, future work will undoubtedly reveal additional mechanisms that utilize the E2 backside to regulate ubiquitination.

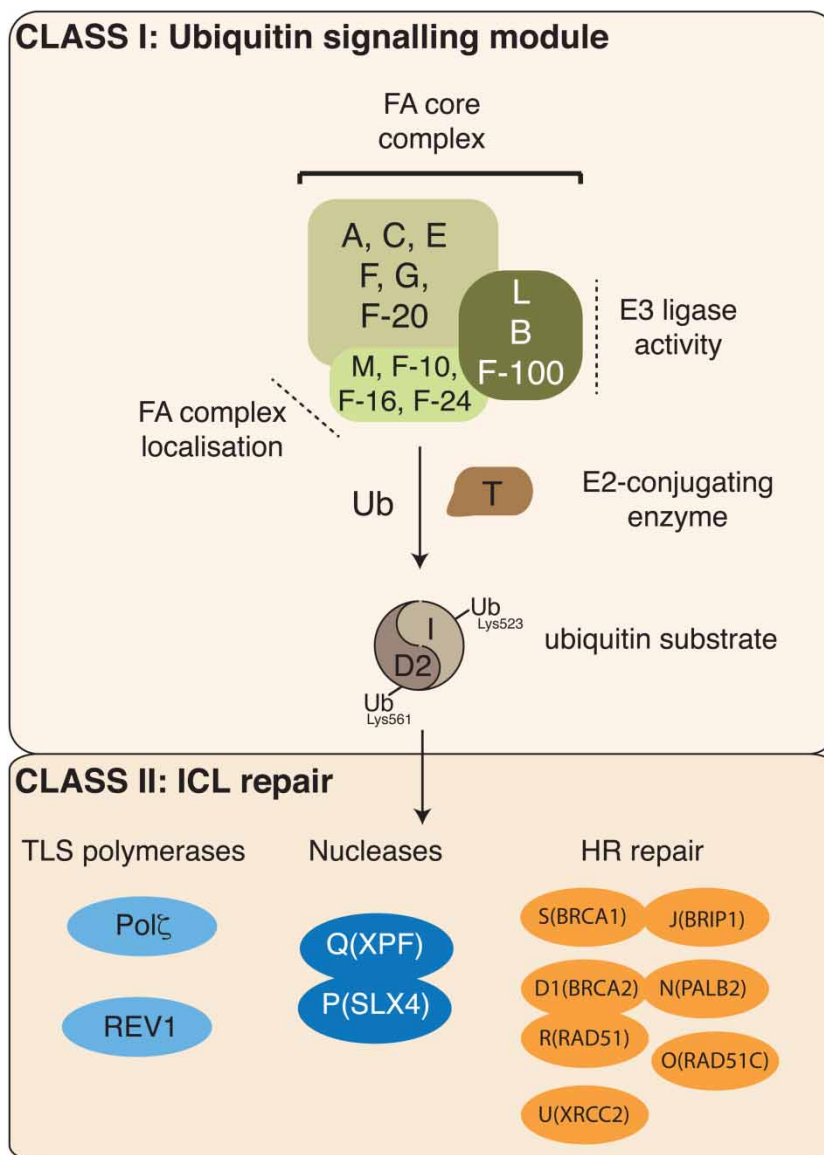
Our current understanding of the mechanisms of E2 action is derived primarily from the extensively studied UBE2D family. However, there are 35 E2s, and an increasing number of them are associated with pathologies and diseases. Therefore, it is not surprising that there is a much greater diversity of action of E2 function than originally expected. Below, we discuss UBE2T and UBE2L3 as specific examples of disease-associated E2s and describe our current understanding of their modes of action.

## **UBE2T (FANCT), the E2 enzyme in the Fanconi anaemia pathway**

### **The Fanconi anaemia ubiquitin signalling module**

An example of the importance of E2 function in disease is the role of UBE2T in the Fanconi anaemia syndrome (FA). The FA DNA repair pathway has become a paradigm for the physiological importance of ubiquitin signalling in coordination of DNA repair pathways and the maintenance of genome stability. In the late 1930s, the Swiss paediatrician Guido Fanconi reported a case of three brothers who all presented with developmental birth defects and eventually died of conditions reminiscent of aplastic anaemia [77]. The syndrome, now known as Fanconi anaemia, was recognized as a rare genetic disorder affecting bone marrow function and haematopoiesis. The combined efforts of many disciplines, ranging from paediatric haematology to biochemistry, determined the syndrome’s molecular defects and uncovered the molecules and mechanism of a complex pathway that is dedicated to repairing DNA interstrand cross-links (ICLs). ICLs are induced after treatment with cross-linking agents such as mitomycin C (MMC) and cisplatin, as well as endogenously by metabolic by-products, most importantly reactive aldehydes. Pioneering work by Patel and colleagues provided compelling evidence of the interplay between aldehyde metabolism and the FA pathway [78–81]. Experiments carried out in mice revealed that increased aldehyde toxicity could trigger bone marrow failure, haematopoietic stem cell depletion, and the development of acute lymphoblastic leukaemia in FA-deficient mice. These defects indeed resemble the key phenotypes of FA patients, and it suggests that the FA pathway has an essential function in neutralizing aldehyde-induced damage in humans.

To date, 20 genes are associated with FA. These genes form two major classes based on their hierarchy and function within the FA pathway (Figure 2). The class I proteins FANCA, -B, -C, -D2, -E, -F, -G, -I, -L, -M, and UBE2T together with the FA-associated proteins (FAAP10, FAAP16, FAAP20, FAAP24, and FAAP100) comprise an ‘upstream’ ubiquitin signalling module. This group co-ordinates ‘downstream’ ICL repair by the class II proteins that include the nuclease scaffold protein SLX4 (FANCP), the structure-specific nuclease XPF (FANCP), and factors that are involved in homologous recombination repair: breast cancer gene 2 (BRCA2;



**Figure 2. The Fanconi anaemia pathway.**

The FA pathway comprising 20 proteins is grouped into two classes: class I defines an upstream ubiquitin signalling module, and class II consists of downstream DNA repair proteins. The FA core complex is activated by stalled replication forks and co-operates with the E2-conjugating enzyme UBE2T (T) to monoubiquitinate the heterodimer FANCI–FANCD2 (I–D2). The RING E3 ligase FANCL (L) is the catalytic subunit, with associated FANCB (B) and FAAP-100 (F-100). Recruitment to stalled forks is mediated by FANCM (M), FAAP-10, -16, and -24. Monoubiquitinated I–D2 then promotes DNA repair by coordinating TLS (translesion synthesis) polymerase, nucleases, and HR-dependent pathways.

FANCD1); PALB2 (FANCN); RAD51C (FANCO); RAD51 (FANCR); XRCC2 (FANCU); BRCA1 (FANCS); and FANCI (BRIP1). The FA genes have been discussed in detail in several excellent reviews [82–85]; hence, we will focus on insights into the FA ubiquitin signalling module, in particular the E2-conjugating enzyme UBE2T.

The first evidence of an ubiquitin signalling module in FA came from the discovery of FANCD2 and its DNA damage-induced monoubiquitination [86]. Monoubiquitinated FANCD2 accumulates on chromatin, forming discrete foci at sites of DNA lesions, co-localizing with DNA repair factors. Any defects in FANCD2 monoubiquitination are as severe as FANCD2 loss-of-function mutations and result in hypersensitivity to



ICL-inducing agents and elevated frequency of chromosomal abnormalities. Thus, it is thought that monoubiquitination is a key step in the signal transduction cascade of the FA pathway. Intriguingly, FANCD2 and the structurally related FA protein FANCI form a stable heterodimer FANCI–FANCD2 (I–D2), and FANCI is the second known substrate for targeted monoubiquitination in the FA pathway [87–89]. Site-specific monoubiquitination of the I–D2 complex on conserved lysine residues (FANCI<sup>Lys523</sup> and FANCD2<sup>Lys561</sup> in humans) is mediated by an E3 ubiquitin ligase, known as the FA core complex.

The FA core complex, first biochemically isolated by Meetei and colleagues, comprises 7 FA gene products FANCA, -B, -C, -E, -F, -G, -L, and the FA-associated proteins FAAP20 and FAAP100 [90–93]. In addition, the core complex associates with the DNA translocase FANCM and FANCM-associated factors FAAP10, -16, and -24 [94–98] and is thought to form a platform to recruit the FA core complex to damaged chromatin [96–98] (Figure 2). Within the FA complex, FANCL is the catalytic RING E3 subunit, and together with FANCB and FAAP100 constitute a minimal E3 module essential for I–D2 monoubiquitination in vertebrate cells [93,99–103].

The identification of the cognate E2-conjugating enzyme for FANCL was the last missing piece in the FA ubiquitin signalling module, as it is currently understood. Using a classical yeast two-hybrid approach, Machida et al. [104] found FANCL to be the predominant interaction partner of UBE2T. RNAi-mediated knockdown of UBE2T in human cells, or deletion of UBE2T in avian DT40 cells, abolished monoubiquitination of FANCD2, suggesting that UBE2T is likely the cognate E2 for FANCL in the catalysis of FANCD2 monoubiquitination [99,104]. This notion was further supported by *in vitro* reconstitution assays, showing that UBE2T and FANCL are necessary and sufficient to monoubiquitinate FANCD2 [100].

The UBE2T enzyme consists of a core UBC fold followed by a C-terminal extension (~40 residues). The extension is largely unstructured, is poorly conserved and has negligible effect in FANCL-mediated FANCD2 monoubiquitination *in vitro* (Arno Alpi and Helen Walden, unpublished observations). A conserved lysine (Lys 91) near the UBE2T catalytic cleft is constitutively monoubiquitinated *in vivo* and has been proposed to negatively regulate the E2 [104]. Interestingly, UBE2T has unusually strong affinity for the FANCL RING domain (~450 nM  $K_d$ ) [103]. Structural characterization of the FANCL RING–UBE2T pair reveals an interface area (~700 Å<sup>2</sup>) that extends beyond the generic E3–E2 interface (typical area — 450 to 600 Å<sup>2</sup>), as well as several features that enable selective interaction for this E3–E2 pair [105]. In particular, an extended hydrophobic interface involves Tyr311 of FANCL anchored within an UBE2T pocket made up of Arg6, Arg9, and Asn103 side chains. This residue is highly variable in other RINGs and typically does not participate in the canonical RING–E2 interface. Furthermore, the β2–β3 loop of UBE2T bears a unique basic residue that forms a salt bridge with Glu340 of FANCL. This residue, Arg60 in UBE2T, is predominantly acidic (Asp/Glu) in other ubiquitin E2s and serves as a positive selector for the FANCL RING–UBE2T pairing.

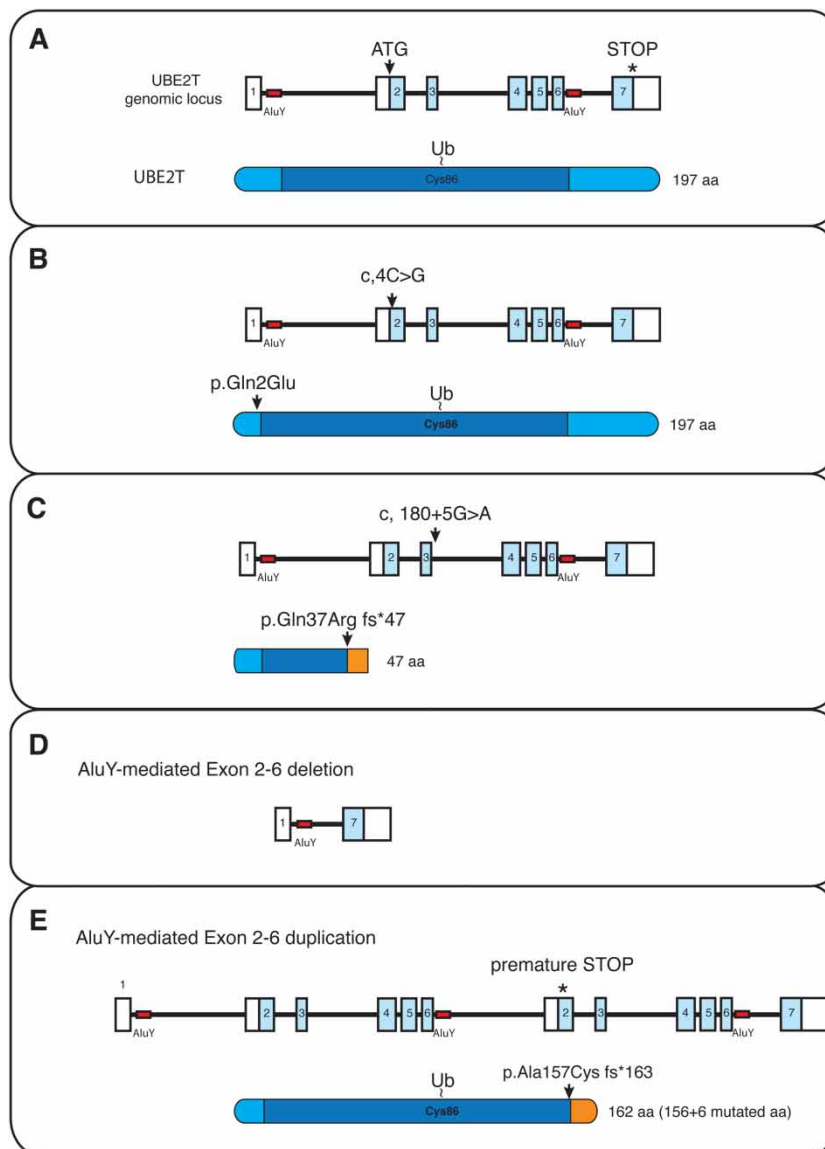
Large-scale E2–E3 interaction studies propose interactions for UBE2T with at least 15 other E3s [18,106–108]. Notably, UBE2T is functional with a limited set of HECT E3s and primarily supports multi-monoubiquitination. However, the proposed UBE2T-binding RING E3s are poorly conserved with the FANCL RING domain; only five of them could possibly support a weak interaction, if any, with the β2–β3 loop in UBE2T. Experimental evidence for the E3s ARIH2 and RING finger 4 (RNF4) shows no significant ubiquitination products with UBE2T [109,110], while reports on a possible UBE2T–BRCA1 RING E3 pair are conflicting [111–113]. Nevertheless, FANCL was able to exclusively complex with UBE2T when presented with a group of different E2s and only the cognate E2–E3 pair resulted in the site-specific monoubiquitination of FANCD2 *in vitro* [105]. The specific and stable FANCL–UBE2T interaction offers insights into how a monoubiquitination event is regulated. As an overlapping UBE2T surface is required for both ubiquitin loading from the E1 and offloading via the E3, the low UBE2T off-rate could limit ubiquitin reloading, and thus curb incessant ubiquitination. However, we still do not completely understand how the FA core complex selects the precise lysine on I–D2 for ubiquitination. In addition, the allosteric ‘linchpin’ residue (typically Arg) required for all RING/U-box-mediated ubiquitination events is absent from FANCL (Ser363) [62]. Future structures of FANCL and/or the FA core complex bound to an UBE2T~Ub intermediate and its substrate will ultimately reveal the determinants of this exquisitely specific ubiquitin signal.

### UBE2T is a *bona fide* FA gene

Almost a decade after the discovery of UBE2T in the FA pathway, three unrelated individuals (PNGS-252, PNGS-255, and 100166/1) diagnosed with FA have been described carrying biallelic mutations in the *UBE2T* gene locus [114–116]. Both PNGS-252 and PNGS-255 patients displayed FA-typical phenotypes including

congenital malformations, haematological abnormalities, and high levels of chromosomal abnormalities that allowed a clear diagnosis. In contrast, 100166/1 was born with developmental abnormalities, but had normal bone marrow and blood counts that are atypical for FA. Despite this, he was eventually diagnosed with FA based on a diepoxybutane analysis revealing high frequencies of DEB(diepoxybutane)-induced chromosomal breakages in haematopoietic cells.

Genome analysis for patient PNGS-252 revealed a maternal c.4C>G missense alteration resulting in the Gln2Glu amino acid substitution and a paternal 23 kb deletion across the *UBE2T* locus (Figure 3A,B) [114]. The Gln2 residue in helix1 of *UBE2T* is not an integral part of the *UBE2T*–FANCL interaction surface.



**Figure 3. Schematic overview of identified *UBE2T* gene alterations in FA patients.**

(A) Genomic locus of *UBE2T* with intron/exon boundaries. Start codon in exon 2, stop codon in exon 7, and AluY repeats (red boxes) are indicated. *UBE2T* encodes a 197 aa protein with catalytic Cys86 in the UBC fold (dark blue). (B) Maternal missense mutation identified in patient PNGS-252 resulting in Gln2 to Glu2 amino acid substitution. (C) The c.180+5G>A splice donor site, identified in patient PNGS-255, initiating a frame shift and premature stop codon resulting in a truncated *UBE2T* protein. Genome alterations identified patient 100166/1 showing the paternal AluY-mediated deletion (D) and maternal AluY-mediated duplication (E). The maternal allele encodes an mRNA for a shorter *UBE2T* protein with a functional UBC fold.

However, the Gln2Glu mutation binds less well to FANCL and reduces FANCD2 monoubiquitination by UBE2T–FANCL *in vitro*. In agreement with this, fibroblasts derived from PNGS-252-expressing mutant UBE2T (Gln2Glu) are defective in ICL-induced FANCD2 monoubiquitination and display MMC hypersensitivity. Individual PNGS-255 had two allelic alterations; the c.4C>G mutation as found in PNGS-252 and, in addition, a c.180+5G>A splice donor site mutation [114]. This latter initiates a frame shift and premature stop codon resulting in a truncated UBE2T protein with a non-functional UBC domain (Figure 3B,C). Both alleles are considered to cause loss of function. The identification of the genome alterations in patient 100166/1 was challenging, as standard sequencing strategies including capillary Sanger and whole exome sequencing did not initially reveal any pathogenic mutations. Ultimately, two large AluY-mediated genome rearrangements were identified [115,116]. The patient inherited from the father a large intragenic deletion in UBE2T resulting from a recombination event between two AluY repeats, creating a new intron between exon 1 and 7. No functional protein is predicted to be expressed as the start codon resides in exon 2 (Figure 3D). Surprisingly, the same AluY repeats appeared to have initiated a recombination in the maternal allele that resulted in the large genomic duplication between these AluY repeats. The maternal allele encodes an mRNA for a truncated, but functional, UBE2T protein. However, the transcript can be detected only at low levels and is degraded by nonsense-mediated mRNA decay due to a premature stop codon (Figure 3E) [116]. Intriguingly, the maternal duplication of exons 2–6 seems to be reverted in the haematopoietic system of the patient and regenerated a wild-type allele, thus explaining that the normal blood counts observed in the patient were due to somatic mosaicism. Despite the fact that AluY repeats are apparently prone to rearrangements in the *UBE2T* genomic locus, further analysis of genomic DNA from normal individuals from Sicily (parents of patient 100166/1 are of Italian origin with ancestors from Sicily), Northern Italy, and Germany did, however, reveal extremely low frequencies of either AluY-mediated deletions or duplications of *UBE2T* exons 2–6 [116]. So far, all identified pathogenic alterations of UBE2T are likely loss-of-function alleles and the deficiency of UBE2T protein is associated with FA. Consequently, 10 years after the discovery of UBE2T protein function, *UBE2T* is now recognized as a *bona fide* FA gene and is alternatively named FANCT.

The specific and selective interaction of UBE2T with FANCL, as well as the typical FA-associated pathologies observed in UBE2T-deficient patients, suggests that UBE2T's primary function is dedicated to monoubiquitinate I–D2 in the FA DNA repair pathway. However, there is some evidence that UBE2T might be associated with ubiquitin signalling pathways other than FA. In contrast with FA core complex-deficient cells (including FANCL deficiency), UBE2T deletion in the avian cell line DT40 (a lymphoblastoid cell) caused hypersensitivity to UV light and cells are less efficient in removing genotoxic cyclobutane pyrimidine photoadducts [117]. Further somatic genetic analysis revealed a genetic link between UBE2T and the nucleotide excision repair gene XPA, suggesting an UBE2T-mediated signalling pathway required for efficient nucleotide excision repair of certain UV lesions. However, the putative E3 ligase and ubiquitin-targeted substrates remain to be identified and are aims for future investigations.

As a historical note, initial evidence suggested UBE2W as an alternative E2 that can associate and function with FANCL to monoubiquitinate FANCD2 [100,118]. However, recent discoveries addressing the genetics and distinct catalytic features of UBE2W argue against a direct function of the E2 in FA and DNA repair pathways. UBE2W contains non-canonical structural characteristics that catalyze monoubiquitin ligation to  $\alpha$ -amino termini by favouring the recognition of substrates with intrinsically disordered N-termini rather than structured epitopes [119]. Thus, the observed monoubiquitination of FANCD2 mediated by UBE2W–FANCL *in vitro* is likely to be an N-terminal ligation and does not recapitulate the lysine site-specific monoubiquitination by UBE2T–FANCL. In agreement with this, deletion of UBE2W in human, mouse, and avian cells does not render them hypersensitive to any tested genotoxins, including ICL-inducing agents [101,120,121], nor is DNA damage-induced FANCD2 monoubiquitination affected [121]. UBE2W-deficient mice are susceptible to early postnatal lethality, show abnormal epidermal differentiation in the skin, and defective immune and male reproductive systems, but none of these pathologies are indicative for FA [120]. Cumulatively, the requirement of UBE2W in the DNA damage response is not essential, but might be part of a more complex regulatory network. Recent work reveals that UBE2W can functionally interact with the RING-type E3 RNF4 [110], which was shown to be essential for DNA double-strand break repair by both homologous recombination and non-homologous end-joining pathways [122,123]. By employing UBE2W, RNF4 is capable of promoting N-terminal monoubiquitination of SUMO chains *in vitro* [110]. However, genetic analysis reveals that simultaneous deletion of UBE2W in RNF4-deficient cells suppresses RNF4-associated DNA repair defects, suggesting

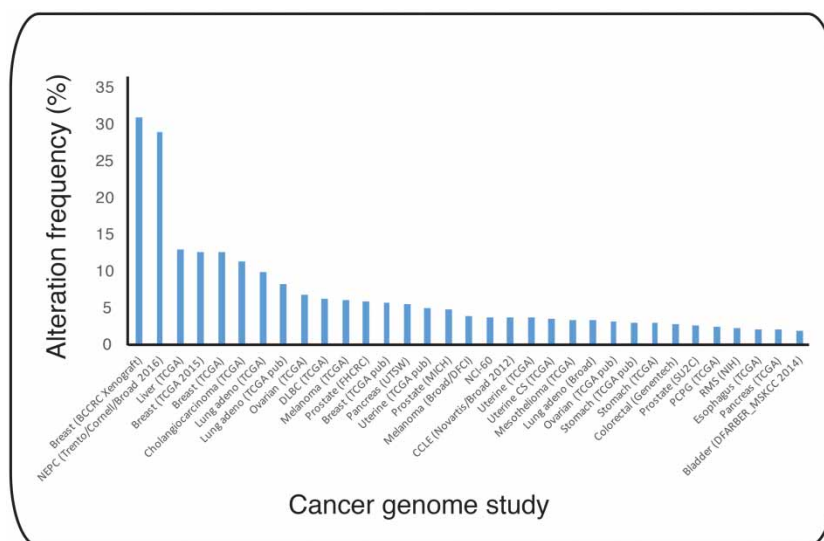


that UBE2W and RNF4 function in different pathways [121]. To date, the biological function of UBE2W is far from being well understood and will be the subject for future investigations.

## UBE2T: potential oncogenic and tumour suppressor function in carcinogenesis

FA patients are highly susceptible to developing some form of malignancy throughout their lives with an observed-to-expected ratio of 48 over all cancers including leukaemia and solid tumours [124]. The majority of described leukaemias are acute myeloid leukaemia (AML), and the most common solid tumours are head and neck squamous cell carcinomas (HNSCC) [124]. FA patients deficient in genes of the homologous recombination repair pathway (FANCD1, FANCN, and FANCF) are particularly prone to breast cancer [125–127], whereas FANCO mutations are associated with increased susceptibility to ovarian cancer [128,129]. So far, an accurate assessment of cancer predisposition linked with UBE2T loss of function is hampered by the limited number of affected patients. Patient PNGS-252 developed severe pancytopenia at age 13, successfully received bone marrow transplantation (BMT), and remains alive in haematological remission since then. Patient PNGS-255 developed AML at age 8. Recovery from a BMT from an HLA-mismatched-related donor was very poor and he died of a severe viral infection soon after [114].

Data mining in large-scale genome analysis and expression profile data from a panel of several hundred cancer cell lines deposited at cBioportal (<http://www.cbioportal.org/>) reveals that many cancers have alterations in UBE2T, in particular high frequencies of amplifications (Figure 4) [130]. Notably, UBE2T locates at 1q32.1, and the gain of 1q is frequently observed in a variety of cancers [131]. Several recent studies investigated UBE2T amplifications and UBE2T as a potential oncogene in different cancers. UBE2T is transcriptionally up-regulated with increased protein levels observed in breast cancers of different histological subtypes, including papillotubular, solid tubular, and scirrhous carcinoma [113]. The potential oncogenic function of elevated UBE2T is likely to be polyubiquitination and subsequent proteasomal degradation of the E3 RING ligase BRCA1 — a primary tumour suppressor associated with mammary carcinogenesis. In contrast, down-regulation of UBE2T by RNAi stabilized BRCA1 and blocked cell growth in breast cancer cells. *In vivo*, it is unclear whether UBE2T forms a direct, productive E2–E3 pair with BRCA1 or indirectly has an impact on the ubiquitin signal by associating with an, as yet unidentified, E3 ligase.



**Figure 4. UBE2T amplifications described in human cancer studies.**

Graphic summary of frequencies of UBE2T alterations in cancers, mainly amplifications based on expression profile data from a panel of cancer cell lines deposited at cBioportal (<http://www.cbioportal.org/>). Frequency of alteration is plotted against data from individual cancer genomic studies. TCGA (The cancer genome atlas), MSKCC (Memorial Sloan-Kettering Cancer Center).

Elevated expression of UBE2T is also associated with lung, gastric, prostate, and nasopharyngeal cancer [132–135]. High UBE2T expression in gastric cancer tissue microarrays correlates with poor prognosis and was proposed as a potential prognostic marker in gastric cancer progression. Ectopic expression of UBE2T in gastric cancer cells promotes cell proliferation and induces epithelial–mesenchymal transition (EMT) by inhibiting the cell-to-cell adhesion factor E-cadherin, but elevating EMT-related factors N-cadherin, P-cadherin, and vimentin levels [135]. Moreover, similar studies of prostate cancer showed that increased UBE2T expression promoted proliferation and was sufficient to induce EMT of prostate cancer cells, as well as enhanced tumour growth in prostate cancer xenograft mouse models and prostate cancer metastasis [133]. Finally, it was demonstrated that UBE2T is highly expressed in nasopharyngeal carcinomas [134]. As in the aforementioned studies, UBE2T overexpression promotes growth of nasopharyngeal carcinoma xenografts and metastasis. Interestingly, elevated UBE2T expression in nasopharyngeal carcinomas activates the AKT/GSK3 $\beta$ / $\beta$ -catenin pathway, and inhibition of the pathway with the AKT inhibitor MK-2206 2HCL blocks the pro-metastatic effect of UBE2T in nasopharyngeal cancer cells [134]. In summary, a common picture emerges from these cancer studies, suggesting that increased UBE2T protein levels and presumed increase in UBE2T activity promote cell transformation and cancer growth. UBE2T is less abundant in cells compared with other E2s, and its expression is tightly controlled [136]. Hence, non-physiological high UBE2T levels may trigger ubiquitination of signal transduction modulators, such as the AKT/GSK3 $\beta$ / $\beta$ -catenin pathway and EMT factors, thereby either altering their activity or their cellular turnover by ubiquitin-target proteasomal degradation. Indeed,  $\beta$ -catenin and vimentin homeostasis is regulated by protein turnover. Given that UBE2T has an essential role in the FA DNA repair pathway, increased UBE2T may also trigger genome instability and subsequently cell transformation. Overall, UBE2T is likely to exhibit diverse roles that are context dependent in different cancer types. Efforts to identify UBE2T-specific substrates should be the ultimate goal in elucidating and dissecting the molecular mechanism of UBE2T in carcinogenesis.

## Disease association of UBE2L3 (UBCH7)

### UBE2L3 (UBCH7) in autoimmune disease

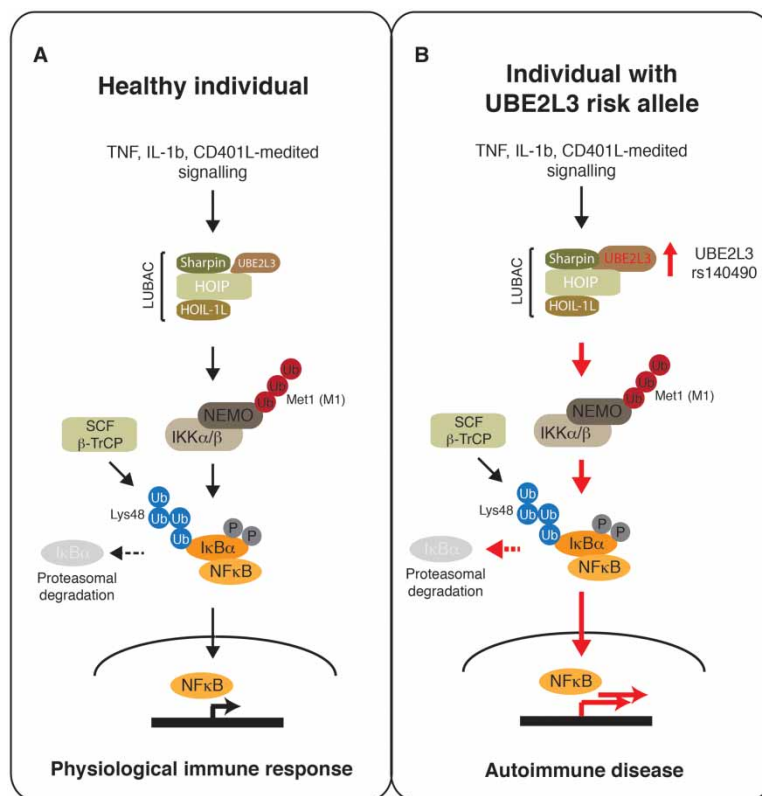
In recent years, several genome-wide association studies (GWAS) identified polymorphisms in the genomic locus of *UBE2L3* that are associated with multiple autoimmune diseases. Haplotype analysis shows that the *UBE2L3* locus has a simple structure encompassing one linkage disequilibrium block covering the majority of genetic variations and extending the full length of the gene locus (summarised in ref. [137]). Several single-nucleotide polymorphisms (SNPs) describe risk alleles associated with systemic lupus erythematosus [138–140], inflammatory bowel disease [141], Crohn's disease [142], rheumatoid arthritis [143], celiac disease [144], psoriasis [145], diffuse cutaneous systemic sclerosis [146], and juvenile idiopathic arthritis [147]. Notably, the risk allele rs5754217 shows significant association with systemic lupus erythematosus and rheumatoid arthritis, suggesting that both autoimmune rheumatological diseases may have *UBE2L3* as a common susceptibility locus [148]. A recent GWAS found that the *UBE2L3* locus is associated with chronic hepatitis virus B infection in Han Chinese patients, suggesting that UBE2L3 is required to clear hepatitis virus B infections. By employing expression quantitative trait locus analysis, the majority of *UBE2L3*-associated SNPs that conferred disease risks correlated with higher expression of UBE2L3. It is tempting to speculate that increased UBE2L3 expression and elevated UBE2L3 activity may be causative of altered immune response pathways triggering autoimmune diseases.

The UBE2L3 enzyme consists of an UBC domain and retains several structural features of the generic UBC fold. However, the catalytic cleft bears a histidine (His119) analogous to the acidic residue in the  $\alpha$ 3– $\alpha$ 4 loop (D117 in UBE2D1) that orients the incoming substrate lysine. Early biochemical and structural studies describe UBE2L3 as predominantly functioning with HECT E3s [149–151]. A landmark study that compared E3-independent E2~Ub reactivity profiles discovered UBE2L3~Ub to be sensitive exclusively to thiol (cysteine) acceptors, whereas UBE2D3~Ub could offload ubiquitin to both free lysines and cysteines [152]. Furthermore, the intrinsic property of UBE2L3 was unaffected by the presence of RING E3s. These data define UBE2L3 as primarily co-operating with HECT or RBR E3 ligases (RING1-BRcat-Rcat E3 ligases), wherein the E2~Ub intermediate offloads ubiquitin onto the E3 catalytic cysteine rather than a substrate lysine.

UBE2L3 surface profiling reveals hot-spot residues (Lys9 in  $\alpha$ 1, Phe63 in the  $\beta$ 2– $\beta$ 3 loop, and Glu93, Lys96, and Lys100 in the  $\beta$ 10-to- $\alpha$ 2 loop) that drive HECT E3 interactions and consequent ubiquitination events [18,153]. Interestingly, the same surface is exploited by the pathogenic *Shigella* effector kinase, OspG, for binding the UBE2L3~Ub intermediate [154,155]. This interaction impedes productive E2–E3 interactions and

downstream ubiquitin signals during innate immune responses while concomitantly enhancing OspG kinase activity that abets pathogenic infection. Recent structure–function studies suggest that HECT/RBR E3s do not require a closed E2~Ub conformation to promote ubiquitination [61–63]. In the case of UBE2L3, the RBR region of HHARI (human homologue of Ariadne) binds tightly with the E2 (roughly 200 nM  $K_d$ ) via an endothermic process [156]. Similar analysis with an activated Parkin E3 species reveals a strong binding with only the UBE2L3~Ub intermediate (roughly 900 nM  $K_d$ ) [157]. Furthermore, kinetic analysis of the interaction between UBE2L3 and the HECT E3 E6-AP (human papillomavirus E6-associated protein) reveals the presence of an, as yet unidentified, E2~Ub docking site that is required for E3~Ub formation, and is distinct from the canonical E3–UBE2L3 interface [158]. Taken together, we are as yet unable to mechanistically define how UBE2L3 undertakes E3~Ub loading as well as inducing polyubiquitination.

The intrinsic catalytic property of UBE2L3 restricts its functional alliance to a subset of E3s, namely the HECT-like E3 ligases and the specialized class of RBR E3s [18,152]. Nevertheless, deep proteomics of *in vivo* protein copy numbers indicate UBE2L3 as one of the most abundant E2s in mammalian cell lines [136]. Importantly, UBE2L3 can form productive E2–E3 pairs with the disease-associated E3 ligase LUBAC *in vitro* [159,160]. The 600 kDa RBR ligase complex LUBAC (linear ubiquitin chain assembly complex) associates with UBE2L3 and specifically forms linear (Met1-linked) ubiquitin chains *in vitro*. LUBAC is composed of HOIL-1L-interacting protein (HOIP), haem-oxidized IRP2 ubiquitin ligase-1 (HOIL-1L), and Sharpin (SHANK-associated RH domain interaction protein in postsynaptic density). Both HOIP and HOIL-1L proteins bear the multidomain signature seen in RBR E3s, but HOIP is thought to be the critical catalytic subunit



**Figure 5. LUBAC and UBE2L3 regulate NF-κB response.**

**(A)** Cytokine/receptor-mediated activation of LUBAC/UBE2L3 triggers linear (Met1) polyubiquitination of NEMO required for IKK kinase activation. IKK phosphorylates the NF-κB sequestration protein IκBα that is then recognized by SCF/β-TrCP ligase for Lys48 polyubiquitination and subsequent degradation by the proteasome. Released NF-κB translocates to the nucleus to transactivate NF-κB response genes. **(B)** Model for hyperactivation (indicated by red arrows) of the NF-κB pathway in patients carrying UBE2L3 risk alleles associated with autoimmune diseases. UBE2L3 SNP rs140490 correlates with increased levels of UBE2L3 protein, causing enhanced LUBAC signalling, accelerated IκBα degradation, and hyperactive NF-κB response.

of LUBAC. The E3 activity of HOIP is latent and is stimulated by heterodimer complex formation with either HOIL-1L or Sharpin. Several lines of genetic evidence suggest an important regulatory role of LUBAC and Met1-chain assembly in immune response signalling cascades (Figure 5A). LUBAC was shown to be critical for the efficient activation of NF- $\kappa$ B signalling, in part, by linear ubiquitination of NEMO, which is required to activate IKK kinase. IKK phosphorylates the NF- $\kappa$ B sequestration protein I $\kappa$ B $\alpha$  and triggers its degradation by the ubiquitin–proteasome system, releasing NF- $\kappa$ B during the initial phase of the NF- $\kappa$ B response. Importantly, recent work suggests that LUBAC participates in TNF receptor-mediated and genotoxin-induced apoptotic pathways by regulating death-promoting complexes in an inhibitory fashion [161–164]. Bi-allelic mutations of HOIL-1L were recently shown to underlie severe autoinflammation, immunodeficiency, and amylopectinosis [165] in human patients. HOIL-1L-deficient fibroblasts, and B-cells derived from patients, display impaired NF- $\kappa$ B activation in response to TLR agonists (TNF and IL-1 $\beta$ ) and CD40L, respectively. In contrast, HOIL-1L-deficient monocytes are hyper-responsive to IL-1 $\beta$ , which probably accounts for the autoinflammatory phenotype in these patients. Recently, a patient with multiorgan autoinflammation and combined immunodeficiency was reported to bear a homozygous mutation in HOIP [166]. The cellular and clinical phenotypes largely overlap with those of HOIL-1L-deficient patients. In summary, there is overwhelming evidence that assembly and activity of LUBAC is required in a cell- and organ-dependent manner for various signalling pathways governing inflammation and immunity.

Given that UBE2L3 and LUBAC are both associated with autoimmune syndromes, an open question remained, until recently, as to whether UBE2L3 is a physiological E2 for LUBAC. Work by Lewis et al. [137] provided some insights into the underlying mechanism of UBE2L3 polymorphism and LUBAC function in systemic lupus erythematosus (Figure 5B). GWAS of systemic lupus erythematosus identified rs140490 as the most strongly associated SNP, which is located downstream of the promoter region of UBE2L3. Messenger RNA for UBE2L3 is increased in CD19<sup>+</sup> B cells and CD4<sup>+</sup> T cells isolated from peripheral blood of individuals with rs140490 genotype. However, rs140490 has increased UBE2L3 protein levels in only CD19<sup>+</sup> B cells. Using NF- $\kappa$ B luciferase reporter cell lines, overexpression of UBE2L3 is not sufficient to increase basal NF- $\kappa$ B activation. However, co-expression of UBE2L3 with HOIP–HOIL-1L or HOIP–Sharpin triggers substantial up-regulation of NF- $\kappa$ B activity, particularly at the late phase of the biphasic NF- $\kappa$ B response. The effect is specific for UBE2L3; other E2s can form productive E2–E3 pairs with LUBAC *in vitro*, such as UBE2D1, UBE2D2, UBE2D3, or UBE2L6, but they do not affect NF- $\kappa$ B activation in the context of LUBAC subcomplexes. Down-regulation of UBE2L3, by siRNA, impairs phosphorylation and degradation of I $\kappa$ B $\alpha$ , similar to that observed in LUBAC-deficient cells [159,167–169]. Hence, UBE2L3 is likely to be the preferred E2 for LUBAC-mediated regulation of the NF- $\kappa$ B response. *Ex vivo* cell experiments demonstrate that the UBE2L3/rs140490 genotype increases basal and stimulated nuclear translocation of NF- $\kappa$ B in primary human B cells and monocytes (Figure 5B). Moreover, analysis of B-cell subsets from human blood samples reveals that the UBE2L3 risk allele is specifically associated with increased levels of UBE2L3 in plasmablasts and plasma cells of systemic lupus erythematosus-affected patients, but not in healthy individuals. Taken together, these data suggest that increased abundance of UBE2L3 and an up-regulated NF- $\kappa$ B response may play a causative role in abnormal B-cell differentiation and proliferation in autoimmune diseases.

### Function of UBE2L3–HOIP in cancer

Hyperactivation of the NF- $\kappa$ B pathway is implicated in several blood cancers [170]. Active B cell-like (ABC) diffuse large B cell lymphomas (DLBCLs) are characterized by the constitutively activated NF- $\kappa$ B pathway, commonly due to gain-of-function mutations in the B-cell receptor and MYD88 pathways. Recently, screening biopsies of ABC-DLBCL have identified rare germline SNPs in the HOIP gene [171]. These SNPs increase the binding of HOIP to HOIL-1L resulting in an increased HOIP activity that contributes to NF- $\kappa$ B hyperactivation in ABC-DLBCL cells. It will be interesting to examine whether UBE2L3 is elevated in ABC-DLBCL cells and whether UBE2L3 is, as proposed by Lewis et al., a rate limiting factor for LUBAC activity. Hence, UBE2L3 might be a potential therapeutic target to treat SLE and B-cell lymphomas that are characterized by LUBAC hyperactivation and constitutive NF- $\kappa$ B signalling.

### Functional association of UBE2L3 with Parkinson’s disease

Genotype and linkage analysis revealed that mutations in the *PARK2* gene, encoding Parkin RBR E3 ligase, are the most frequent cause of sporadic early- and late-onset Parkinson’s disease (PD) cases [172,173]. In addition, *PARK2* was described as a putative tumour suppressor gene [174]. Parkin is thought to have an essential

neuroprotective function by regulating mitophagy required for mitochondrial homeostasis and quality control. However, the identity of the cognate E2 that associates with Parkin in mitophagy and PD is less well defined. Parkin E3 ligase activity is auto-inhibited by the N-terminal UBL domain, which obscures the catalytic interaction between Parkin and the ubiquitin-loaded E2 [175–178]. Parkin's auto-inhibited state is relieved, and E3 ligase activated, by a dual PINK1 kinase-mediated phosphorylation event that involves phosphorylation of a crucial residue Ser65 on the UBL domain as well as binding of phospho-Ser65 ubiquitin [179–183]. Subsequently, PINK1-activated Parkin allows the interaction with different ubiquitin-loaded E2s, including UBE2L3, to trigger and amplify ubiquitin signals at damaged mitochondria. Recent studies suggest a potential role of UBE2L3 in Parkin-dependent mitophagy [184,185]. Initial evidence came from *in situ* hybridization studies in rat brain tissues that indicate similar pattern and developmental changes in the expression of Parkin and UBE2L3 [186]. UBE2L3 is translocated to depolarized mitochondria dependent on active Parkin [184]. siRNA-mediated knockdown of UBE2L3 does not interfere with Parkin translocation, but impairs Parkin-dependent clearance of depolarized mitochondria [184,185]. Interestingly, only the simultaneous knockdown of UBE2L3, UBE2N, and UBE2D2/3 reduces ubiquitination of proteins of depolarized mitochondria [184]. Therefore, Parkin apparently employs different E2 enzymes to generate distinct ubiquitin chain signals that mediate efficient mitophagy. The apparent redundant functions of UBE2L3 with other E2s may explain the current lack of any genetic association of *UBE2L3* gene alterations with PD.

## Future perspectives

Our current understanding of E2 function includes substantial insights into the mechanisms common to all E2s. This field is now entering a phase of uncovering the differences between the enzymes that position each E2 in a given physiological context. This may also provide us with a framework to explain pathological functions of E2s associated with diseases. Indeed, the spectrum of pathologies and diseases associated with E2s is broad, ranging from immunological disorders and neurological syndromes (e.g. UBE2A [187]) to cancer, and undoubtedly, further involvement of E2s in other diseases will be discovered. The majority of E2s are linked with cancer of many different origins. The functional roles of several E2s in breast carcinogenesis have been described (reviewed in ref. [188]). For example, UBE2N is an emerging key player in various cancers, and the recently developed small compound inhibitor NSC697923 targeting UBE2N inhibits proliferation and survival of neuroblastoma and DLBCL cells. With the advent of enzymatic tools for trapping various states of E2 intermediates, there are now more opportunities to address the unique properties of E2s in different biological contexts and, importantly, to dissect disease pathways [189,190]. Certainly, this will pave the way to exploit E2s in therapeutic contexts, in particular by developing new strategies to target individual E2s or specific interactions of E2–E3 pairs.

## Abbreviations

AKT alias PKB, protein kinase B; AML, acute myeloid leukaemia; BARD1, BRCA1 associated RING domain protein 1; BMT, bone marrow transplantation; BRCA1, breast cancer gene 1; BRcat, benign required for catalysis; DEP, diepoxybutane; DLBCL, diffuse large B cell lymphomas; DUB, deubiquitylating enzyme; E6-AP, human papillomavirus E6-associated protein; EMT, epithelial–mesenchymal transition; eQTL, expression quantitative trait locus; FA, Fanconi anaemia; FAAP, FA-associated protein; GSK3, glycogen synthase kinase 3; GWSA, genome-wide association study; HECT, homologous to the E6-AP C-terminus; HHARI, human homolog of ariadne; HNSCC, head and neck squamous cell carcinomas; HOIL-1, haem-oxidized IRP2 ubiquitin ligase-1; HOIP, HOIL-1L interacting protein; ICL, inter-strand cross-link; IKK, I $\kappa$ B (inhibitor kappa B) kinase; LUBAC, linear ubiquitin chain assembly complex; MMC, mitomycin C; NEDD8, neural precursor cell expressed developmentally down-regulated protein 8; NEMO, nuclear factor  $\kappa$ B essential modulator; PCGF, polycomb group RING finger; PCNA, proliferating cell nuclear antigen; PD, Parkinson's disease; PTM, post-translational modification; RBR E3 ligases, RING1–BRcat–Rcat E3 ligases; Rcat, required for catalysis; RING, really interesting new gene; RNF, RING finger; SNP, single nucleotide polymorphism; SHARPIN, shank-associated RH domain-interacting protein; SLE, systemic lupus erythematosus; SUMO, small ubiquitin-like modifier; TLR, Toll-like receptor; TRIM, tripartite motif containing protein; Ub, ubiquitin; UBC, ubiquitin conjugation; UBD, ubiquitin-binding domain; UFD, ubiquitin-fold domain.

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## Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

## References

- 1 Pickart, C.M. (2001) Mechanisms underlying ubiquitination. *Annu. Rev. Biochem.* **70**, 503–533 doi:10.1146/annurev.biochem.70.1.503
- 2 Sloper-Mould, K.E., Jemc, J.C., Pickart, C.M. and Hicke, L. (2001) Distinct functional surface regions on ubiquitin. *J. Biol. Chem.* **276**, 30483–30489 doi:10.1074/jbc.M103248200
- 3 Kulathu, Y. and Komander, D. (2012) Atypical ubiquitylation—the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat. Rev. Mol. Cell Biol.* **13**, 508–523 doi:10.1038/nrm3394
- 4 Alfano, C., Faggiano, S. and Pastore, A. (2016) The ball and chain of polyubiquitin structures. *Trends Biochem. Sci.* **41**, 371–385 doi:10.1016/j.tibs.2016.01.006
- 5 Husnjak, K. and Dikic, I. (2012) Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annu. Rev. Biochem.* **81**, 291–322 doi:10.1146/annurev-biochem-051810-094654
- 6 Clague, M.J., Barsukov, I., Coulson, J.M., Liu, H., Rigden, D.J. and Urbe, S. (2013) Deubiquitylases from genes to organism. *Physiol. Rev.* **93**, 1289–1315 doi:10.1152/physrev.00002.2013
- 7 Enchev, R.I., Schulman, B.A. and Peter, M. (2014) Protein neddylation: beyond cullin-RING ligases. *Nat. Rev. Mol. Cell Biol.* **16**, 30–44 doi:10.1038/nrm3919
- 8 Flotho, A. and Melchior, F. (2013) Sumoylation: a regulatory protein modification in health and disease. *Annu. Rev. Biochem.* **82**, 357–385 doi:10.1146/annurev-biochem-061909-093311
- 9 Heideker, J. and Wertz, I.E. (2015) DUBs, the regulation of cell identity and disease. *Biochem. J.* **465**, 1–26 doi:10.1042/BJ20140496
- 10 Charan, R.A. and LaVoie, M.J. (2015) Pathologic and therapeutic implications for the cell biology of parkin. *Mol. Cell. Neurosci.* **66**(Pt A), 62–71 doi:10.1016/j.mcn.2015.02.008
- 11 Roy, R., Chun, J. and Powell, S.N. (2011) BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat. Rev. Cancer* **12**, 68–78 doi:10.1038/nrc3181
- 12 Hatakeyama, S. (2011) TRIM proteins and cancer. *Nat. Rev. Cancer* **11**, 792–804 doi:10.1038/nrc3139
- 13 van Wijk, S.J. and Timmers, H.T. (2010) The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. *FASEB J.* **24**, 981–993 doi:10.1096/fj.09-136259
- 14 Burroughs, A.M., Jaffee, M., Iyer, L.M. and Aravind, L. (2008) Anatomy of the E2 ligase fold: implications for enzymology and evolution of ubiquitin/Ub-like protein conjugation. *J. Struct. Biol.* **162**, 205–218 doi:10.1016/j.jsb.2007.12.006
- 15 Michelle, C., Vourc'h, P., Mignon, L. and Andres, C.R. (2009) What was the set of ubiquitin and ubiquitin-like conjugating enzymes in the eukaryote common ancestor? *J. Mol. Evol.* **68**, 616–628 doi:10.1007/s00239-009-9225-6
- 16 Berndsen, C.E., Wiener, R., Yu, I.W., Ringel, A.E. and Wolberger, C. (2013) A conserved asparagine has a structural role in ubiquitin-conjugating enzymes. *Nat. Chem. Biol.* **9**, 154–156 doi:10.1038/nchembio.1159
- 17 Cook, B.W. and Shaw, G.S. (2012) Architecture of the catalytic HPN motif is conserved in all E2 conjugating enzymes. *Biochem. J.* **445**, 167–174 doi:10.1042/BJ20120504
- 18 Sheng, Y., Hong, J.H., Doherty, R., Srikumar, T., Shloush, J., Avakumov, G.V. et al. (2012) A human ubiquitin conjugating enzyme (E2)-HECT E3 ligase structure-function screen. *Mol. Cell Proteomics* **11**, 329–341 doi:10.1074/mcp.0111.013706
- 19 Plechanovová, A., Jaffray, E.G., Tatham, M.H., Naismith, J.H. and Hay, R.T. (2012) Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. *Nature* **489**, 115–120 doi:10.1038/nature11376
- 20 Sarcevic, B., Mawson, A., Baker, R.T. and Sutherland, R.L. (2002) Regulation of the ubiquitin-conjugating enzyme hHR6A by CDK-mediated phosphorylation. *EMBO J.* **21**, 2009–2018 doi:10.1093/emboj/21.8.2009
- 21 Shchebet, A., Karpiuk, O., Kremmer, E., Eick, D. and Johnsen, S.A. (2012) Phosphorylation by cyclin-dependent kinase-9 controls ubiquitin-conjugating enzyme-2A function. *Cell Cycle* **11**, 2122–2127 doi:10.4161/cc.20548
- 22 Valimberti, I., Tiberti, M., Lambrugh, M., Sarcevic, B. and Papaleo, E. (2015) E2 superfamily of ubiquitin-conjugating enzymes: constitutively active or activated through phosphorylation in the catalytic cleft. *Sci. Rep.* **5**, 14849 doi:10.1038/srep14849
- 23 Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2005) The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. *Mol. Cell* **20**, 589–599 doi:10.1016/j.molcel.2005.09.010
- 24 Chiu, Y.-H., Sun, Q. and Chen, Z.J. (2007) E1-L2 activates both ubiquitin and FAT10. *Mol. Cell.* **27**, 1014–1023 doi:10.1016/j.molcel.2007.08.020
- 25 Jin, J., Li, X., Gygi, S.P. and Harper, J.W. (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**, 1135–1138 doi:10.1038/nature05902
- 26 Pelzer, C., Kassner, I., Matentzoglou, K., Singh, R.K., Wollscheid, H.-P., Scheffner, M. et al. (2007) UBE1L2, a novel E1 enzyme specific for ubiquitin. *J. Biol. Chem.* **282**, 23010–23014 doi:10.1074/jbc.C700111200
- 27 Schulman, B.A. and Harper, J.W. (2009) Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat. Rev. Mol. Cell Biol.* **10**, 319–331 doi:10.1038/nrm2673
- 28 Streich, Jr, F.C. and Lima, C.D. (2014) Structural and functional insights to ubiquitin-like protein conjugation. *Annu. Rev. Biophys.* **43**, 357–379 doi:10.1146/annurev-biophys-051013-022958
- 29 Hamilton, K.S., Ellison, M.J., Barber, K.R., Williams, R.S., Huzil, J.T., McKenna, S. et al. (2001) Structure of a conjugating enzyme-ubiquitin thiolester intermediate reveals a novel role for the ubiquitin tail. *Structure* **9**, 897–904 doi:10.1016/S0969-2126(01)00657-8
- 30 Page, R.C., Pruneda, J.N., Amick, J., Klevit, R.E. and Misra, S. (2012) Structural insights into the conformation and oligomerization of E2~ubiquitin conjugates. *Biochemistry* **51**, 4175–4187 doi:10.1021/bi300058m
- 31 Pruneda, J.N., Stoll, K.E., Bolton, L.J., Brzovic, P.S. and Klevit, R.E. (2011) Ubiquitin in motion: structural studies of the ubiquitin-conjugating enzyme approximately ubiquitin conjugate. *Biochemistry* **50**, 1624–1633 doi:10.1021/bi101913m
- 32 Saha, A., Lewis, S., Kleiger, G., Kuhlman, B. and Deshaies, R.J. (2011) Essential role for ubiquitin-ubiquitin-conjugating enzyme interaction in ubiquitin discharge from Cdc34 to substrate. *Mol. Cell* **42**, 75–83 doi:10.1016/j.molcel.2011.03.016

- 33 Wickliffe, K.E., Lorenz, S., Wemmer, D.E., Kuriyan, J. and Rape, M. (2011) The mechanism of linkage-specific ubiquitin chain elongation by a single-subunit E2. *Cell* **144**, 769–781 doi:10.1016/j.cell.2011.01.035
- 34 Rodrigo-Brenni, M.C., Foster, S.A. and Morgan, D.O. (2010) Catalysis of lysine 48-specific ubiquitin chain assembly by residues in E2 and ubiquitin. *Mol. Cell* **39**, 548–559 doi:10.1016/j.molcel.2010.07.027
- 35 Middleton, A.J. and Day, C.L. (2015) The molecular basis of lysine 48 ubiquitin chain synthesis by Ube2K. *Sci. Rep.* **5**, 16793 doi:10.1038/srep16793
- 36 Lorenz, S., Bhattacharyya, M., Feiler, C., Rape, M. and Kuriyan, J. (2016) Crystal structure of a Ube2S-Ubiquitin conjugate. *PLoS ONE* **11**, e0147550 doi:10.1371/journal.pone.0147550
- 37 Eddins, M.J., Carlile, C.M., Gomez, K.M., Pickart, C.M. and Wolberger, C. (2006) Mms2-Ubc13 covalently bound to ubiquitin reveals the structural basis of linkage-specific polyubiquitin chain formation. *Nat. Struct. Mol. Biol.* **13**, 915–920 doi:10.1038/nsmb1148
- 38 Li, W., Tu, D., Brunger, A.T. and Ye, Y. (2007) A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. *Nature* **446**, 333–337 doi:10.1038/nature05542
- 39 Ravid, T. and Hochstrasser, M. (2007) Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. *Nat. Cell Biol.* **9**, 422–427 doi:10.1038/ncb1558
- 40 Liu, W., Shang, Y., Zeng, Y., Liu, C., Li, Y., Zhai, L. et al. (2014) Dimeric Ube2g2 simultaneously engages donor and acceptor ubiquitins to form Lys48-linked ubiquitin chains. *EMBO J.* **33**, 46–61 doi:10.1002/emboj.201385315
- 41 Petroski, M.D. and Deshaies, R.J. (2005) Mechanism of lysine 48-linked ubiquitin-chain synthesis by the cullin-RING ubiquitin-ligase complex SCF-Cdc34. *Cell* **123**, 1107–1120 doi:10.1016/j.cell.2005.09.033
- 42 Chong, R.A., Wu, K., Spratt, D.E., Yang, Y., Lee, C., Nayak, J. et al. (2014) Pivotal role for the ubiquitin Y59-E51 loop in lysine 48 polyubiquitination. *Proc. Natl Acad. Sci. USA* **111**, 8434–8439 doi:10.1073/pnas.1407849111
- 43 Ziemba, A., Hill, S., Sandoval, D., Webb, K., Bennett, E.J. and Kleiger, G. (2013) Multimodal mechanism of action for the Cdc34 acidic loop: a case study for why ubiquitin-conjugating enzymes have loops and tails. *J. Biol. Chem.* **288**, 34882–34896 doi:10.1074/jbc.M113.509190
- 44 Stewart, M.D., Ritterhoff, T., Klevit, R.E. and Brzovic, P.S. (2016) E2 enzymes: more than just middle men. *Cell Res.* **26**, 423–440 doi:10.1038/cr.2016.35
- 45 Windheim, M., Peggie, M. and Cohen, P. (2008) Two different classes of E2 ubiquitin-conjugating enzymes are required for the mono-ubiquitination of proteins and elongation by polyubiquitin chains with a specific topology. *Biochem. J.* **409**, 723–729 doi:10.1042/BJ20071338
- 46 Parker, J.L. and Ulrich, H.D. (2009) Mechanistic analysis of PCNA poly-ubiquitylation by the ubiquitin protein ligases Rad18 and Rad5. *EMBO J.* **28**, 3657–3666 doi:10.1038/emboj.2009.303
- 47 Rodrigo-Brenni, M.C. and Morgan, D.O. (2007) Sequential E2s drive polyubiquitin chain assembly on APC targets. *Cell* **130**, 127–139 doi:10.1016/j.cell.2007.05.027
- 48 Schumacher, F.-R., Wilson, G. and Day, C.L. (2013) The N-terminal extension of UBE2E ubiquitin-conjugating enzymes limits chain assembly. *J. Mol. Biol.* **425**, 4099–4111 doi:10.1016/j.jmb.2013.06.039
- 49 Sun, L. and Fang, J. (2016) E3-independent constitutive monoubiquitination complements histone methyltransferase activity of SETDB1. *Mol. Cell* **62**, 958–966 doi:10.1016/j.molcel.2016.04.022
- 50 Nakada, S., Tai, I., Panier, S., Al-Hakim, A., Iemura, S.-I., Juang, Y.C. et al. (2010) Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. *Nature* **466**, 941–946 doi:10.1038/nature09297
- 51 Wiener, R., DiBello, A.T., Lombardi, P.M., Guzzo, C.M., Zhang, X., Matunis, M.J. et al. (2013) E2 ubiquitin-conjugating enzymes regulate the deubiquitinating activity of OTUB1. *Nat. Struct. Mol. Biol.* **20**, 1033–1039 doi:10.1038/nsmb.2655
- 52 Juang, Y.-C., Landry, M.-C., Sanches, M., Vittal, V., Leung, C.C.Y., Ceccarelli, D.F. et al. (2012) OTUB1 co-opts Lys48-linked ubiquitin recognition to suppress E2 enzyme function. *Mol. Cell* **45**, 384–397 doi:10.1016/j.molcel.2012.01.011
- 53 Eletr, Z.M., Huang, D.T., Duda, D.M., Schulman, B.A. and Kuhlman, B. (2005) E2 conjugating enzymes must disengage from their E1 enzymes before E3-dependent ubiquitin and ubiquitin-like transfer. *Nat. Struct. Mol. Biol.* **12**, 933–934 doi:10.1038/nsmb984
- 54 Huang, D.T., Paydar, A., Zhuang, M., Waddell, M.B., Holton, J.M. and Schulman, B.A. (2005) Structural basis for recruitment of Ubc12 by an E2 binding domain in NEDD8's E1. *Mol. Cell.* **17**, 341–350 doi:10.1016/j.molcel.2004.12.020
- 55 Benirschke, R.C., Thompson, J.R., Nominé, Y., Wasielewski, E., Juranić, N., Macura, S. et al. (2010) Molecular basis for the association of human E4B U box ubiquitin ligase with E2-conjugating enzymes UbcH5c and Ubc4. *Structure* **18**, 955–965 doi:10.1016/j.str.2010.04.017
- 56 Xu, Z., Kohli, E., Devlin, K.I., Bold, M., Nix, J.C. and Misra, S. (2008) Interactions between the quality control ubiquitin ligase CHIP and ubiquitin conjugating enzymes. *BMC Struct. Biol.* **8**, 26 doi:10.1186/1472-6807-8-26
- 57 Mace, P.D., Linke, K., Feltham, R., Schumacher, F.-R., Smith, C.A., Vaux, D.L. et al. (2008) Structures of the cAP2 RING domain reveal conformational changes associated with ubiquitin-conjugating enzyme (E2) recruitment. *J. Biol. Chem.* **283**, 31633–31640 doi:10.1074/jbc.M804753200
- 58 Metzger, M.B., Pruneda, J.N., Klevit, R.E. and Weissman, A.M. (2014) RING-type E3 ligases: master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. *Biochim. Biophys. Acta* **1843**, 47–60 doi:10.1016/j.bbamcr.2013.05.026
- 59 Scheffner, M. and Kumar, S. (2014) Mammalian HECT ubiquitin-protein ligases: biological and pathophysiological aspects. *Biochim. Biophys. Acta* **1843**, 61–74 doi:10.1016/j.bbamcr.2013.03.024
- 60 Spratt, D.E., Walden, H. and Shaw, G.S. (2014) RBR e3 ubiquitin ligases: new structures, new insights, new questions. *Biochem. J.* **458**, 421–437 doi:10.1042/BJ20140006
- 61 Kamadurai, H.B., Souphron, J., Scott, D.C., Duda, D.M., Miller, D.J., Stringer, D. et al. (2009) Insights into ubiquitin transfer cascades from a structure of a UbcH5B approximately ubiquitin-HECT(NEDD4L) complex. *Mol. Cell* **36**, 1095–1102 doi:10.1016/j.molcel.2009.11.010
- 62 Pruneda, J.N., Littlefield, P.J., Soss, S.E., Nordquist, K.A., Chazin, W.J., Brzovic, P.S. et al. (2012) Structure of an E3:E2~Ub Complex Reveals an Allosteric Mechanism Shared among RING/U-box Ligases. *Mol. Cell* **47**, 933–942 doi:10.1016/j.molcel.2012.07.001
- 63 Lechtenberg, B.C., Rajput, A., Sanishvili, R., Dobaczewska, M.K., Ware, C.F., Mace, P.D. et al. (2016) Structure of a HOIP/E2~ubiquitin complex reveals RBR E3 ligase mechanism and regulation. *Nature* **529**, 546–550 doi:10.1038/nature16511
- 64 Dove, K.K., Stieglitz, B., Duncan, E.D., Rittinger, K. and Klevit, R.E. (2016) Molecular insights into RBR E3 ligase ubiquitin transfer mechanisms. *EMBO Rep* **17**, 1221–1235 doi:10.15252/embr.201642641

- 65 Dou, H., Buetow, L., Sibbet, G.J., Cameron, K. and Huang, D.T. (2012) BIRC7-E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. *Nat. Struct. Mol. Biol.* **19**, 876–883 doi:10.1038/nsmb.2379
- 66 Dou, H., Buetow, L., Sibbet, G.J., Cameron, K. and Huang, D.T. (2013) Essentiality of a non-RING element in priming donor ubiquitin for catalysis by a monomeric E3. *Nat. Struct. Mol. Biol.* **20**, 982–986 doi:10.1038/nsmb.2621
- 67 McGinty, R.K., Henrici, R.C. and Tan, S. (2014) Crystal structure of the PRC1 ubiquitylation module bound to the nucleosome. *Nature* **514**, 591–596 doi:10.1038/nature13890
- 68 Brzovic, P.S., Lissounov, A., Christensen, D.E., Hoyt, D.W. and Klevit, R.E. (2006) A UbcH5/ubiquitin noncovalent complex is required for processive BRCA1-directed ubiquitination. *Mol. Cell* **21**, 873–880 doi:10.1016/j.molcel.2006.02.008
- 69 Buetow, L., Gabrielsen, M., Anthony, N.G., Dou, H., Patel, A., Aitkenhead, H. et al. (2015) Activation of a primed RING E3-E2-ubiquitin complex by non-covalent ubiquitin. *Mol. Cell* **58**, 297–310 doi:10.1016/j.molcel.2015.02.017
- 70 Das, R., Mariano, J., Tsai, Y.C., Kalathur, R.C., Kostova, Z., Li, J. et al. (2009) Allosteric activation of E2-RING finger-mediated ubiquitylation by a structurally defined specific E2-binding region of gp78. *Mol. Cell* **34**, 674–685 doi:10.1016/j.molcel.2009.05.010
- 71 Li, W., Tu, D., Li, L., Wollert, T., Ghirlando, R., Brunger, A.T. et al. (2009) Mechanistic insights into active site-associated polyubiquitination by the ubiquitin-conjugating enzyme Ubc2g2. *Proc. Natl Acad. Sci. USA* **106**, 3722–3727 doi:10.1073/pnas.0808564106
- 72 Das, R., Liang, Y.-H., Mariano, J., Li, J., Huang, T., King, A. et al. (2013) Allosteric regulation of E2:E3 interactions promote a processive ubiquitination machine. *EMBO J.* **32**, 2504–2516 doi:10.1038/emboj.2013.174
- 73 Metzger, M.B., Liang, Y.-H., Das, R., Mariano, J., Li, S., Li, J. et al. (2013) A structurally unique E2-binding domain activates ubiquitination by the ERAD E2, Ubc7p, through multiple mechanisms. *Mol. Cell* **50**, 516–527 doi:10.1016/j.molcel.2013.04.004
- 74 Li, S., Liang, Y.H., Mariano, J., Metzger, M.B., Stringer, D.K., Hristova, V.A. et al. (2015) Insights into ubiquitination from the unique clamp-like binding of the RING E3 A07 to the E2 UbcH5B. *J. Biol. Chemistry* **290**, 30225–30239 doi:10.1074/jbc.M115.685867
- 75 Hibbert, R.G., Huang, A., Boelens, R. and Sixma, T.K. (2011) E3 ligase Rad18 promotes monoubiquitination rather than ubiquitin chain formation by E2 enzyme Rad6. *Proc. Natl Acad. Sci. USA* **108**, 5590–5595 doi:10.1073/pnas.1017516108
- 76 Turco, E., Gallego, L.D., Schneider, M. and Köhler, A. (2015) Monoubiquitination of histone H2B is intrinsic to the Bre1 RING domain-Rad6 interaction and augmented by a second Rad6-binding site on Bre1. *J. Biol. Chem.* **290**, 5298–5310 doi:10.1074/jbc.M114.626788
- 77 Lobitz, S. and Velleuer, E. (2006) Guido Fanconi (1892–1979): a jack of all trades. *Nat. Rev. Cancer* **6**, 893–898 doi:10.1038/nrc2009
- 78 Langevin, F., Crossan, G.P., Rosado, I.V., Arends, M.J. and Patel, K.J. (2011) Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. *Nature* **475**, 53–58 doi:10.1038/nature10192
- 79 Rosado, I.V., Langevin, F., Crossan, G.P., Takata, M. and Patel, K.J. (2011) Formaldehyde catabolism is essential in cells deficient for the Fanconi anemia DNA-repair pathway. *Nat. Struct. Mol. Biol.* **18**, 1432–1434 doi:10.1038/nsmb.2173
- 80 Pontel, L.B., Rosado, I.V., Burgos-Barragan, G., Garaycochea, J.I., Yu, R., Arends, M.J. et al. (2015) Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. *Mol. Cell* **60**, 177–188 doi:10.1016/j.molcel.2015.08.020
- 81 Garaycochea, J.I., Crossan, G.P., Langevin, F., Daly, M., Arends, M.J. and Patel, K.J. (2012) Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function. *Nature* **489**, 571–575 doi:10.1038/nature11368
- 82 Bogliolo, M. and Surrallés, J. (2015) Fanconi anemia: a model disease for studies on human genetics and advanced therapeutics. *Curr. Opin. Genet. Dev.* **33**, 32–40 doi:10.1016/j.gde.2015.07.002
- 83 Duxin, J.P. and Walter, J.C. (2015) What is the DNA repair defect underlying Fanconi anemia?? *Curr. Opin. Cell Biol.* **37**, 49–60 doi:10.1016/j.ccb.2015.09.002
- 84 Lopez-Martinez, D., Liang, C.-C. and Cohn, M.A. (2016) Cellular response to DNA interstrand crosslinks: the Fanconi anemia pathway. *Cell. Mol. Life Sci.* **73**, 3097–3114 doi: 10.1007/s00018-016-2218-x
- 85 Ceccaldi, R., Sarangi, P. and D'Andrea, A.D. (2016) The Fanconi anaemia pathway: new players and new functions. *Nat. Rev. Mol. Cell Biol.* **17**, 337–349 doi:10.1038/nrm.2016.48
- 86 Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M.S., Timmers, C., Hejna, J. et al. (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* **7**, 249–262 doi:10.1016/S1097-2765(01)00173-3
- 87 Smogorzewska, A., Matsuoka, S., Vinciguerra, P., McDonald, III, E.R., Hurov, K.E., Luo, J. et al. (2007) Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* **129**, 289–301 doi:10.1016/j.cell.2007.03.009
- 88 Sims, A.E., Spiteri, E., Sims, III, R.J., Arita, A.G., Lach, F.P., Landers, T. et al. (2007) FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nat. Struct. Mol. Biol.* **14**, 564–567 doi:10.1038/nsmb1252
- 89 Dorsman, J.C., Levitus, M., Rockx, D., Rooimans, M.A., Oostra, A.B., Haitjema, A. et al. (2007) Identification of the Fanconi anemia complementation group I gene, FANCI. *Cell. Oncol.* **29**, 211–218 PMID:17452773
- 90 Meetei, A.R., Sechi, S., Wallisch, M., Yang, D., Young, M.K., Joenje, H. et al. (2003) A multiprotein nuclear complex connects Fanconi anemia and Bloom syndrome. *Mol. Cell. Biol.* **23**, 3417–3426 doi:10.1128/MCB.23.10.3417-3426.2003
- 91 Ling, C., Ishiai, M., Ali, A.M., Medhurst, A.L., Neveling, K., Kalb, R. et al. (2007) FAAP100 is essential for activation of the Fanconi anemia-associated DNA damage response pathway. *EMBO J.* **26**, 2104–2114 doi:10.1038/sj.emboj.7601666
- 92 Ali, A.M., Pradhan, A., Singh, T.R., Du, C., Li, J., Wahengbam, K. et al. (2012) FAAP20: a novel ubiquitin-binding FA nuclear core-complex protein required for functional integrity of the FA-BRCA DNA repair pathway. *Blood* **119**, 3285–3294 doi:10.1182/blood-2011-10-385963
- 93 Meetei, A.R., de Winter, J.P., Medhurst, A.L., Wallisch, M., Waisfisz, Q., van de Vrugt, H.J. et al. (2003) A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat. Genet.* **35**, 165–170 doi:10.1038/ng1241
- 94 Meetei, A.R., Medhurst, A.L., Ling, C., Xue, Y., Singh, T.R., Bier, P. et al. (2005) A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat. Genet.* **37**, 958–963 doi:10.1038/ng1626
- 95 Mosedale, G., Niedzwiedz, W., Alpi, A., Perrina, F., Pereira-Leal, J.B., Johnson, M. et al. (2005) The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat. Struct. Mol. Biol.* **12**, 763–771 doi:10.1038/nsmb981
- 96 Ciccio, A., Ling, C., Coulthard, R., Yan, Z., Xue, Y., Meetei, A.R. et al. (2007) Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol. Cell* **25**, 331–343 doi:10.1016/j.molcel.2007.01.003

- 97 Singh, T.R., Saro, D., Ali, A.M., Zheng, X.-F., Du, C.-H., Killen, M.W. et al. (2010) MHF1-MHF2, a histone-fold-containing protein complex, participates in the Fanconi anemia pathway via FANCM. *Mol. Cell* **37**, 879–886 doi:10.1016/j.molcel.2010.01.036
- 98 Yan, Z., Delannoy, M., Ling, C., Dae, D., Osman, F., Muniandy, P.A. et al. (2010) A histone-fold complex and FANCM form a conserved DNA-remodeling complex to maintain genome stability. *Mol. Cell* **37**, 865–878 doi:10.1016/j.molcel.2010.01.039
- 99 Alpi, A., Langevin, F., Mosedale, G., Machida, Y.J., Dutta, A. and Patel, K.J. (2007) UBE2T, the Fanconi anemia core complex, and FANCD2 are recruited independently to chromatin: a basis for the regulation of FANCD2 monoubiquitination. *Mol. Cell Biol.* **27**, 8421–8430 doi:10.1128/MCB.00504-07
- 100 Alpi, A.F., Pace, P.E., Babu, M.M. and Patel, K.J. (2008) Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol. Cell* **32**, 767–777 doi:10.1016/j.molcel.2008.12.003
- 101 Rajendra, E., Oestergaard, V.H., Langevin, F., Wang, M., Dornan, G.L., Patel, K.J. et al. (2014) The genetic and biochemical basis of FANCD2 monoubiquitination. *Mol. Cell* **54**, 858–869 doi:10.1016/j.molcel.2014.05.001
- 102 Cole, A.R., Lewis, L.P.C. and Walden, H. (2010) The structure of the catalytic subunit FANCL of the Fanconi anemia core complex. *Nat. Struct. Mol. Biol.* **17**, 294–298 doi:10.1038/nsmb.1759
- 103 Hodson, C., Cole, A.R., Lewis, L.P.C., Miles, J.A., Purkiss, A. and Walden, H. (2011) Structural analysis of human FANCL, the E3 ligase in the Fanconi anemia pathway. *J. Biol. Chem.* **286**, 32628–32637 doi:10.1074/jbc.M111.244632
- 104 Machida, Y.J., Machida, Y., Chen, Y., Gurtan, A.M., Kupfer, G.M., D'Andrea, A.D. et al. (2006) UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol. Cell* **23**, 589–596 doi:10.1016/j.molcel.2006.06.024
- 105 Hodson, C., Purkiss, A., Miles, J.A. and Walden, H. (2014) Structure of the human FANCL RING-Ube2T complex reveals determinants of cognate E3-E2 selection. *Structure* **22**, 337–344 doi:10.1016/j.str.2013.12.004
- 106 Kar, G., Keskin, O., Nussinov, R. and Gursoy, A. (2012) Human proteome-scale structural modeling of E2-E3 interactions exploiting interface motifs. *J. Proteome Res.* **11**, 1196–1207 doi:10.1021/pr2009143
- 107 Markson, G., Kiel, C., Hyde, R., Brown, S., Charalabous, P., Bremm, A. et al. (2009) Analysis of the human E2 ubiquitin conjugating enzyme protein interaction network. *Genome Res.* **19**, 1905–1911 doi:10.1101/gr.093963.109
- 108 van Wijk, S.J., de Vries, S.J., Kemmeren, P., Huang, A., Boelens, R., Bonvin, A.M. et al. (2009) A comprehensive framework of E2-RING E3 interactions of the human ubiquitin-proteasome system. *Mol. Syst. Biol.* **5**, 295 doi:10.1038/msb.2009.76
- 109 Kelsall, I.R., Duda, D.M., Olszewski, J.L., Hofmann, K., Knebel, A., Langevin, F. et al. (2013) TRIAD1 and HHARI bind to and are activated by distinct neddylated Cullin-RING ligase complexes. *EMBO J.* **32**, 2848–2860 doi:10.1038/emboj.2013.209
- 110 Tatham, M.H., Plechanová, A., Jaffray, E.G., Salmen, H. and Hay, R.T. (2013) Ube2w conjugates ubiquitin to alpha-amino groups of protein N-termini. *Biochem. J.* **453**, 137–145 doi:10.1042/BJ20130244
- 111 Christensen, D.E., Brzovic, P.S. and Klevit, R.E. (2007) E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. *Nat. Struct. Mol. Biol.* **14**, 941–948 doi:10.1038/nsmb1295
- 112 Christensen, D.E. and Klevit, R.E. (2009) Dynamic interactions of proteins in complex networks: identifying the complete set of interacting E2s for functional investigation of E3-dependent protein ubiquitination. *FEBS J.* **276**, 5381–5389 doi:10.1111/j.1742-4658.2009.07249.x
- 113 Ueki, T., Park, J.-H., Nishidate, T., Kijima, K., Hirata, K., Nakamura, Y. et al. (2009) Ubiquitination and downregulation of BRCA1 by ubiquitin-conjugating enzyme E2T overexpression in human breast cancer cells. *Cancer Res.* **69**, 8752–8760 doi:10.1158/0008-5472.CAN-09-1809
- 114 Hira, A., Yoshida, K., Sato, K., Okuno, Y., Shiraiishi, Y., Chiba, K. et al. (2015) Mutations in the gene encoding the E2 conjugating enzyme UBE2T cause Fanconi anemia. *Am. J. Hum. Genet.* **96**, 1001–1007 doi:10.1016/j.ajhg.2015.04.022
- 115 Rickman, K.A., Lach, F.P., Abhyankar, A., Donovan, F.X., Sanborn, E.M., Kennedy, J.A. et al. (2015) Deficiency of UBE2T, the E2 ubiquitin ligase necessary for FANCD2 and FANCL ubiquitination, causes FA-T subtype of Fanconi anemia. *Cell. Rep.* **12**, 35–41 doi:10.1016/j.celrep.2015.06.014
- 116 Virts, E.L., Jankowska, A., Mackay, C., Glaas, M.F., Wiek, C., Kelich, S.L. et al. (2015) AluY-mediated germline deletion, duplication and somatic stem cell reversion in *UBE2T* defines a new subtype of Fanconi anemia. *Hum. Mol. Genet.* **24**, 5093–5108 doi:10.1093/hmg/ddv227
- 117 Kelsall, I.R., Langenick, J., Mackay, C., Patel, K.J. and Alpi, A.F. (2012) The Fanconi anaemia components UBE2T and FANCM are functionally linked to nucleotide excision repair. *PLoS ONE* **7**, e36970 doi:10.1371/journal.pone.0036970
- 118 Zhang, Y., Zhou, X., Zhao, L., Li, C., Zhu, H., Xu, L. et al. (2011) UBE2W interacts with FANCL and regulates the monoubiquitination of Fanconi anemia protein FANCD2. *Mol. Cells* **31**, 113–122 doi:10.1007/s10059-011-0015-9
- 119 Vittal, V., Shi, L., Wenzel, D.M., Scaglione, K.M., Duncan, E.D., Basrur, V. et al. (2014) Intrinsic disorder drives N-terminal ubiquitination by Ube2w. *Nat. Chem. Biol.* **11**, 83–89 doi:10.1038/nchembio.1700
- 120 Wang, B., Merillat, S.A., Vincent, M., Huber, A.K., Basrur, V., Mangelberger, D. et al. (2016) Loss of the ubiquitin-conjugating enzyme UBE2W results in susceptibility to early postnatal lethality and defects in skin, immune, and Male reproductive systems. *J. Biol. Chem.* **291**, 3030–3042 doi:10.1074/jbc.M115.676601
- 121 Maure, J.F., Moser, S.C., Jaffray, E.G., Alpi, A.F. and Hay, R.T. (2016) Loss of ubiquitin E2 Ube2w rescues hypersensitivity of Rnf4 mutant cells to DNA damage. *Sci. Rep.* **6**, 26178 doi:10.1038/srep26178
- 122 Yin, Y., Seifert, A., Chua, J.S., Maure, J.-F., Golebiowski, F. and Hay, R.T. (2012) SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes Dev.* **26**, 1196–1208 doi:10.1101/gad.189274.112
- 123 Galanty, Y., Belotserkovskaya, R., Coates, J. and Jackson, S.P. (2012) RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* **26**, 1179–1195 doi:10.1101/gad.188284.112
- 124 Alter, B.P. (2014) Fanconi anemia and the development of leukemia. *Best. Pract. Res. Clin. Haematol.* **27**, 214–221 doi:10.1016/j.beha.2014.10.002
- 125 Wooster, R., Neuhausen, S.L., Mangion, J., Quirk, Y., Ford, D., Collins, N. et al. (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science* **265**, 2088–2090 doi:10.1126/science.8091231
- 126 Rahman, N., Seal, S., Thompson, D., Kelly, P., Renwick, A., Elliott, A. et al. (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat. Genet.* **39**, 165–167 doi:10.1038/ng1959
- 127 Cantor, S.B., Bell, D.W., Ganesan, S., Kass, E.M., Drapkin, R., Grossman, S. et al. (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* **105**, 149–160 doi:10.1016/S0092-8674(01)00304-X



- 128 Loveday, C., Turnbull, C., Ruark, E., Xicola, R.M., Ramsay, E., Hughes, D. et al. (2012) Germline RAD51C mutations confer susceptibility to ovarian cancer. *Nat. Genet.* **44**, 475–476; author reply 6 doi:10.1038/ng.2224
- 129 Meindl, A., Hellebrand, H., Wiek, C., Erven, V., Wappenschmidt, B., Niederacher, D. et al. (2010) Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat. Genet.* **42**, 410–414 doi:10.1038/ng.569
- 130 Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O. et al. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 doi:10.1126/scisignal.2004088
- 131 Corson, T.W., Huang, A., Tsao, M.-S. and Gallie, B.L. (2005) KIF14 is a candidate oncogene in the 1q minimal region of genomic gain in multiple cancers. *Oncogene* **24**, 4741–4753 doi:10.1038/sj.onc.1208641
- 132 Hao, J., Xu, A., Xie, X., Hao, J., Tian, T., Gao, S. et al. (2008) Elevated expression of UBE2T in lung cancer tumors and cell lines. *Tumour Biol.* **29**, 195–203 doi:10.1159/000148187
- 133 Wen, M., Kwon, Y., Wang, Y., Mao, J.-H. and Wei, G. (2015) Elevated expression of UBE2T exhibits oncogenic properties in human prostate cancer. *Oncotarget* **6**, 25226–25239 doi:10.18632/oncotarget.4712
- 134 Hu, W., Xiao, L., Cao, C., Hua, S. and Wu, D. (2016) UBE2T promotes nasopharyngeal carcinoma cell proliferation, invasion, and metastasis by activating the AKT/GSK3beta/beta-catenin pathway. *Oncotarget* **7**, 15161–15172 PMID:26943030
- 135 Yu, H., Xiang, P., Pan, Q., Huang, Y., Xie, N. and Zhu, W. (2016) Ubiquitin-conjugating enzyme E2T is an independent prognostic factor and promotes gastric cancer progression. *Tumour Biol* 1–10 doi:10.1007/s13277-016-5020-3
- 136 Clague, M.J., Heride, C. and Urbé, S. (2015) The demographics of the ubiquitin system. *Trends Cell Biol.* **25**, 417–426 doi:10.1016/j.tcb.2015.03.002
- 137 Lewis, M.J., Vyse, S., Shields, A.M., Boeltz, S., Gordon, P.A., Spector, T.D. et al. (2015) UBE2L3 polymorphism amplifies NF-κB activation and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases. *Am. J. Hum. Genet.* **96**, 221–234 doi:10.1016/j.ajhg.2014.12.024
- 138 Han, J.-W., Zheng, H.-F., Cui, Y., Sun, L.-D., Ye, D.-Q., Hu, Z. et al. (2009) Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat. Genet.* **41**, 1234–1237 doi:10.1038/ng.472
- 139 Gateva, V., Sandling, J.K., Hom, G., Taylor, K.E., Chung, S.A., Sun, X. et al. (2009) A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat. Genet.* **41**, 1228–1233 doi:10.1038/ng.468
- 140 Wang, S., Adrianto, I., Wiley, G.B., Lessard, C.J., Kelly, J.A., Adler, A.J. et al. (2012) A functional haplotype of UBE2L3 confers risk for systemic lupus erythematosus. *Genes Immun.* **13**, 380–387 doi:10.1038/gene.2012.6
- 141 Jostins, L., Ripke, S., Weersma, R.K., Duerr, R.H., McGovern, D.P., Hui, K.Y. et al. (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 doi:10.1038/nature11582
- 142 Franke, A., McGovern, D.P., Barrett, J.C., Wang, K., Radford-Smith, G.L., Ahmad, T. et al. (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat. Genet.* **42**, 1118–1125 doi:10.1038/ng.717
- 143 Stahl, E.A., Raychaudhuri, S., Remmers, E.F., Xie, G., Eyre, S., Thomson, B.P. et al. (2010) Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* **42**, 508–514 doi:10.1038/ng.582
- 144 Dubois, P.C., Trynka, G., Franke, L., Hunt, K.A., Romanos, J., Curtotti, A. et al. (2010) Multiple common variants for celiac disease influencing immune gene expression. *Nat. Genet.* **42**, 295–302 doi:10.1038/ng.543
- 145 Tsoi, L.C., Spain, S.L., Knight, J., Ellinghaus, E., Stuart, P.E., Capon, F. et al. (2012) Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* **44**, 1341–1348 doi:10.1038/ng.2467
- 146 Hasebe, N., Kawasaki, A., Ito, I., Kawamoto, M., Hasegawa, M., Fujimoto, M. et al. (2012) Association of UBE2L3 polymorphisms with diffuse cutaneous systemic sclerosis in a Japanese population. *Ann. Rheum. Dis.* **71**, 1259–1260 doi:10.1136/annrheumdis-2011-201091
- 147 Hinks, A., Cobb, J., Marion, M.C., Prahald, S., Sudman, M., Bowes, J. et al. (2013) Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat. Genet.* **45**, 664–669 doi:10.1038/ng.2614
- 148 Orozco, G., Eyre, S., Hinks, A., Bowes, J., Morgan, A.W., Wilson, A.G. et al. (2011) Study of the common genetic background for rheumatoid arthritis and systemic lupus erythematosus. *Ann. Rheum. Dis.* **70**, 463–468 doi:10.1136/ard.2010.137174
- 149 Huang, L., Kinnucan, E., Wang, G., Beaudenon, S., Howley, P.M., Huibregtse, J.M. et al. (1999) Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade. *Science* **286**, 1321–1326 doi:10.1126/science.286.5443.1321
- 150 Kumar, S., Kao, W.H. and Howley, P.M. (1997) Physical interaction between specific E2 and Hect E3 enzymes determines functional cooperativity. *J. Biol. Chem.* **272**, 13548–13554 doi:10.1074/jbc.272.21.13548
- 151 Nuber, U. and Scheffner, M. (1999) Identification of determinants in E2 ubiquitin-conjugating enzymes required for hect E3 ubiquitin-protein ligase interaction. *J. Biol. Chem.* **274**, 7576–7582 doi:10.1074/jbc.274.11.7576
- 152 Wenzel, D.M., Lissounov, A., Brzovic, P.S. and Klevit, R.E. (2011) UBCH7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature* **474**, 105–108 doi:10.1038/nature09966
- 153 Eletr, Z.M. and Kuhlman, B. (2007) Sequence determinants of E2-E6AP binding affinity and specificity. *J. Mol. Biol.* **369**, 419–428 doi:10.1016/j.jmb.2007.03.026
- 154 Grishin, A.M., Condos, T.E., Barber, K.R., Campbell-Valois, F.X., Parsot, C., Shaw, G.S. et al. (2014) Structural basis for the inhibition of host protein ubiquitination by Shigella effector kinase OspG. *Structure* **22**, 878–888 doi:10.1016/j.str.2014.04.010
- 155 Kim, D.W., Lenzen, G., Page, A.-L., Legrain, P., Sansonetti, P.J. and Parsot, C. (2005) The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc. Natl Acad. Sci. USA* **102**, 14046–14051 doi:10.1073/pnas.0504466102
- 156 Duda, D.M., Olszewski, J.L., Schuermann, J.P., Kurinov, I., Miller, D.J., Nourse, A. et al. (2013) Structure of HHARI, a RING-IBR-RING ubiquitin ligase: autoinhibition of an Ariadne-family E3 and insights into ligation mechanism. *Structure* **21**, 1030–1041 doi:10.1016/j.str.2013.04.019
- 157 Kumar, A., Aguirre, J.D., Condos, T.E., Martinez-Torres, R.J., Chaugule, V.K., Toth, R. et al. (2015) Disruption of the autoinhibited state primes the E3 ligase parkin for activation and catalysis. *EMBO J.* **34**, 2506–2521 doi:10.15252/emj.201592337
- 158 Ronchi, V.P., Klein, J.M. and Haas, A.L. (2013) E6AP/UBE3A ubiquitin ligase harbors two E2 ubiquitin binding sites. *J. Biol. Chem.* **288**, 10349–10360 doi:10.1074/jbc.M113.458059
- 159 Kirisako, T., Kamei, K., Murata, S., Kato, M., Fukumoto, H., Kanie, M. et al. (2006) A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J.* **25**, 4877–4887 doi:10.1038/sj.emboj.7601360



- 160 Stieglitz, B., Morris-Davies, A.C., Koliopoulos, M.G., Christodoulou, E. and Rittinger, K. (2012) LUBAC synthesizes linear ubiquitin chains via a thioester intermediate. *EMBO Rep.* **13**, 840–846 doi:10.1038/embor.2012.105
- 161 Rickard, J.A., Anderton, H., Etemadi, N., Nachbur, U., Darding, M., Peltzer, N. et al. (2014) TNFR1-dependent cell death drives inflammation in *Sharpin*-deficient mice. *eLife* **3** doi:10.7554/eLife.03464
- 162 Kumari, S., Redouane, Y., Lopez-Mosqueda, J., Shiraishi, R., Romanowska, M., Lutzmayer, S. et al. (2014) Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *eLife* **3** doi:10.7554/eLife.03422
- 163 Peltzer, N., Rieser, E., Taraborrelli, L., Draber, P., Darding, M., Pernaute, B. et al. (2014) HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. *Cell Rep.* **9**, 153–165 doi:10.1016/j.celrep.2014.08.066
- 164 Mackay, C., Carroll, E., Ibrahim, A.F., Garg, A., Inman, G.J., Hay, R.T. et al. (2014) E3 ubiquitin ligase HOIP attenuates apoptotic cell death induced by cisplatin. *Cancer Res.* **74**, 2246–2257 doi:10.1158/0008-5472.CAN-13-2131
- 165 Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z. et al. (2012) Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat. Immunol.* **13**, 1178–1186 doi:10.1038/ni.2457
- 166 Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M. et al. (2015) Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J. Exp. Med.* **212**, 939–951 doi:10.1084/jem.20141130
- 167 Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M. et al. (2011) SHARPIN forms a linear ubiquitin ligase complex regulating NF- $\kappa$ B activity and apoptosis. *Nature* **471**, 637–641 doi:10.1038/nature09814
- 168 Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S. et al. (2011) SHARPIN is a component of the NF- $\kappa$ B-activating linear ubiquitin chain assembly complex. *Nature* **471**, 633–636 doi:10.1038/nature09815
- 169 Gertlach, B., Cordier, S.M., Schmukle, A.C., Emmerich, C.H., Rieser, E., Haas, T.L. et al. (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591–596 doi:10.1038/nature09816
- 170 Perkins, N.D. (2012) The diverse and complex roles of NF- $\kappa$ B subunits in cancer. *Nat. Rev. Cancer* **12**, 121–132 doi:10.1038/nrc3204
- 171 Yang, Y., Schmitz, R., Mitala, J., Whiting, A., Xiao, W., Ceribelli, M. et al. (2014) Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. *Cancer Discov.* **4**, 480–493 doi:10.1158/2159-8290.CD-13-0915
- 172 Dawson, T.M. and Dawson, V.L. (2010) The role of parkin in familial and sporadic Parkinson's disease. *Mov. Disord.* **25**(Suppl 1), S32–S39 doi:10.1002/mds.22798
- 173 Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S. et al. (1998) Mutations in the parkin gene cause autosomal recessive juvenile Parkinsonism. *Nature* **392**, 605–608 doi:10.1038/33416
- 174 Devine, M.J., Plun-Favreau, H. and Wood, N.W. (2011) Parkinson's disease and cancer: two wars, one front. *Nat. Rev. Cancer* **11**, 812–823 doi:10.1038/nrc3150
- 175 Chaugule, V.K., Burchell, L., Barber, K.R., Sidhu, A., Leslie, S.J., Shaw, G.S. et al. (2011) Autoregulation of Parkin activity through its ubiquitin-like domain. *EMBO J.* **30**, 2853–2867 doi:10.1038/emboj.2011.204
- 176 Spratt, D.E., Julio Martinez-Torres, R., Noh, Y.J., Mercier, P., Manczyk, N., Barber, K.R. et al. (2013) A molecular explanation for the recessive nature of parkin-linked Parkinson's disease. *Nat. Commun.* **4**, 1983 doi:10.1038/ncomms2983
- 177 Trempe, J.F., Sauve, V., Grenier, K., Seirafi, M., Tang, M.Y., Menade, M. et al. (2013) Structure of Parkin reveals mechanisms for ubiquitin ligase activation. *Science* **340**, 1451–1455 doi:10.1126/science.1237908
- 178 Wauer, T. and Komander, D. (2013) Structure of the human Parkin ligase domain in an autoinhibited state. *EMBO J.* **32**, 2099–112 doi:10.1038/emboj.2013.125
- 179 Kondapalli, C., Kazlauskaitė, A., Zhang, N., Woodroof, H.I., Campbell, D.G., Gourlay, R. et al. (2012) PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating serine 65. *Open Biol.* **2**, 120080 doi:10.1098/rsob.120080
- 180 Iguchi, M., Kujuro, Y., Okatsu, K., Koyano, F., Kosako, H., Kimura, M. et al. (2013) Parkin-catalyzed ubiquitin-ester transfer is triggered by PINK1-dependent phosphorylation. *J. Biol. Chem.* **288**, 22019–22032 doi:10.1074/jbc.M113.467530
- 181 Kane, L.A., Lazarou, M., Fogel, A.I., Li, Y., Yamano, K., Sarraf, S.A. et al. (2014) PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J. Cell Biol.* **205**, 143–153 doi:10.1083/jcb.201402104
- 182 Kazlauskaitė, A., Kondapalli, C., Gourlay, R., Campbell, D.G., Ritoro, M.S., Hofmann, K. et al. (2014) Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem. J.* **460**, 127–141 doi:10.1042/BJ20140334
- 183 Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M. et al. (2014) Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* **510**, 162–166 doi:10.1038/nature13392
- 184 Geisler, S., Vollmer, S., Golombek, S. and Kahle, P.J. (2014) The ubiquitin-conjugating enzymes UBE2N, UBE2L3 and UBE2D2/3 are essential for Parkin-dependent mitophagy. *J. Cell. Sci.* **127**(Pt 15), 3280–3293 doi:10.1242/jcs.146035
- 185 Fiesel, F.C., Moussaoud-Lamodiere, E.L., Ando, M. and Springer, W. (2014) A specific subset of E2 ubiquitin-conjugating enzymes regulate Parkin activation and mitophagy differently. *J. Cell Sci.* **127**(Pt 16), 3488–3504 doi:10.1242/jcs.147520
- 186 Wang, M., Suzuki, T., Kitada, T., Asakawa, S., Minoshima, S., Shimizu, N. et al. (2001) Developmental changes in the expression of parkin and UbcR7, a parkin-interacting and ubiquitin-conjugating enzyme, in rat brain. *J. Neurochem.* **77**, 1561–1568 doi:10.1046/j.1471-4159.2001.00372.x
- 187 Nascimento, R.M., Otto, P.A., de Brouwer, A.P. and Vianna-Morgante, A.M. (2006) UBE2A, which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome. *Am. J. Hum. Genet.* **79**, 549–555 doi:10.1086/507047
- 188 Voutsadakis, I.A. (2013) Ubiquitin- and ubiquitin-like proteins-conjugating enzymes (E2s) in breast cancer. *Mol. Biol. Rep.* **40**, 2019–2034 doi:10.1007/s11033-012-2261-0
- 189 Mulder, M.P., Witting, K., Berlin, I., Pruneda, J.N., Wu, K.P., Chang, J.G. et al. (2016) A cascading activity-based probe sequentially targets E1-E2-E3 ubiquitin enzymes. *Nat. Chem. Biol.* **12**, 523–530 doi:10.1038/nchembio.2084
- 190 Pao, K.-C., Stanley, M., Han, C., Lai, Y.-C., Murphy, P., Balk, K. et al. (2016) Probes of ubiquitin E3 ligases enable systematic dissection of parkin activation. *Nat. Chem. Biol.* **12**, 324–331 doi:10.1038/nchembio.2045