

# Structural basis of ubiquitylation

Andrew P VanDemark and Christopher P Hill\*

The attachment of the small protein ubiquitin to other proteins, a process known as ubiquitylation, is a widespread form of post-translational modification that regulates numerous cellular functions in eukaryotes. Ubiquitylation is performed by complexes of E2 and E3 enzymes that are assembled and select substrates via a series of protein–protein interactions. Recent structure determinations of the ubiquitylation machinery have revealed some of the various protein–protein interfaces involved.

## Addresses

Department of Biochemistry, 20N 1900E RM 211, University of Utah, Salt Lake City, UT 84132, USA

\*e-mail: chris@biochem.utah.edu

Current Opinion in Structural Biology 2002, 12:822–830

0959-440X/02/\$ – see front matter

© 2002 Elsevier Science Ltd. All rights reserved.

## Abbreviations

APC	anaphase promoting complex
Hyp	hydroxyproline
LRR	leucine-rich repeat
SH	Src homology
UEV	ubiquitin E2 variant

## Introduction

Ubiquitin is a small, 76 amino acid protein that packs a powerful punch. Covalent attachment of the ubiquitin C terminus to substrate lysine residues, a process known as ubiquitylation, targets the substrate for a range of possible fates, the best known of which is degradation by the 26S proteasome, but which also include endocytosis, targeting to lysosomes, and modification of protein function [1]. These responses to ubiquitylation play a critical role in regulating fundamental cellular processes, including metabolic homeostasis, protein quality control, transcription, translation, signal transduction, response to hypoxia, cell cycle progression, DNA repair, protein trafficking, and viral budding. As usual, evolution has copied and modified a successful scheme, in this case to generate ubiquitin-like proteins that share structural and functional similarities with ubiquitin, including parallel biochemical pathways for post-translational attachment to lysine sidechains of substrate proteins [2].

The biochemical process of ubiquitylation [3] starts with activation of ubiquitin by an ATP-dependent E1 enzyme, followed by transfer to the cysteine sidechain of an E2 enzyme (also called Ubc), with which the ubiquitin C terminus forms a thiolester (Figure 1a). The ubiquitin C terminus is then ligated through an isopeptide bond to a substrate lysine by an E2–E3 complex. In many cases, the ubiquitin is itself ubiquitylated; for example, most proteasomal substrates are targeted by their attachment to polyubiquitin chains in which Lys48 and the C terminus of successive ubiquitin entities are covalently connected [4].

A major cellular effort is committed to the process of ubiquitylation, with numerous E2 and E3 protein subunits identified in yeast and higher eukaryotes. Two mechanistically distinct families of E3 exist. HECT domain E3s (Figure 1b) transfer ubiquitin to the substrate via the formation of a covalent ubiquitin–E3 thiolester intermediate [5], whereas the RING E3s promote the transfer of ubiquitin from E2 directly to the substrate [6]. The large number of RING E3s can be further categorized on the basis of their overall architecture, whereby either the RING domain is embedded within a larger polypeptide chain that also encodes the substrate recognition domain (Figure 1c) or distinct RING and other subunits are organized as a multi-subunit complex on a cullin protein scaffold (Figure 1d) [7,8]. Figure 2 shows a gallery of recently determined structures, including large fragments of the HECT domain E3 E6-AP and the RING domain E3 c-Cbl, and various subcomplexes of the RING subunit E3s SCF and VCB.

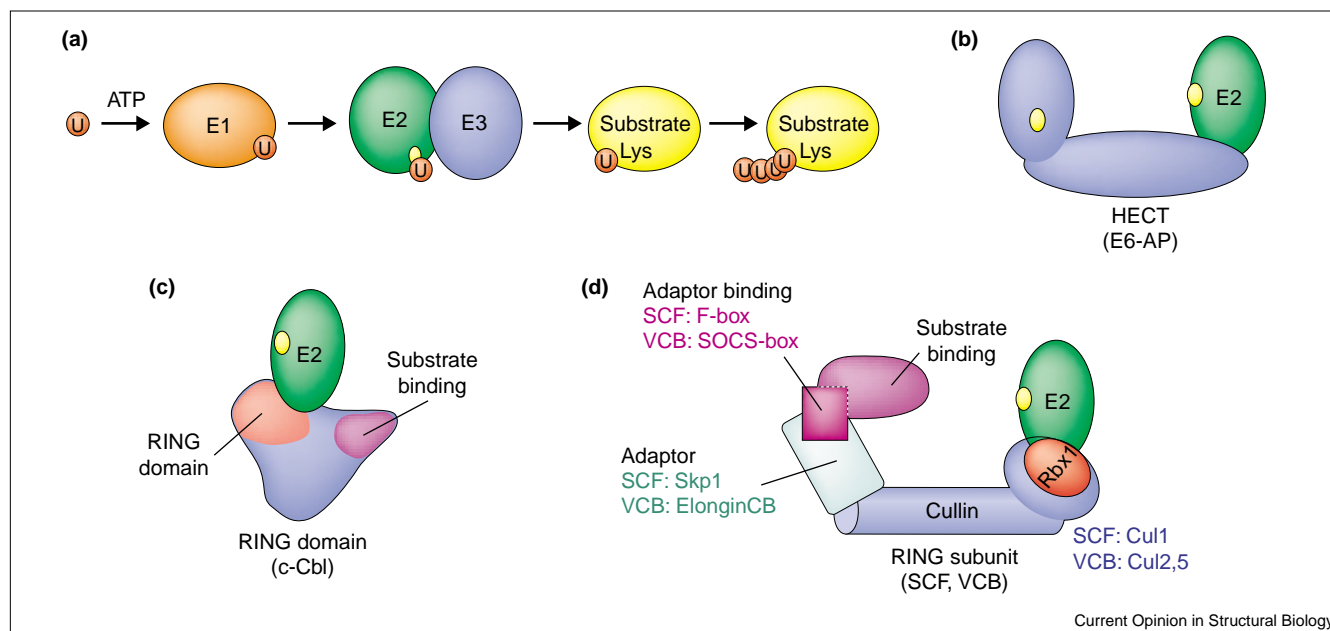
## E2–E3 interface

E2 enzymes have a ~150-residue core domain that often comprises the entire protein, but may also include N- or C-terminal extensions. Several E2 structures have been determined, all of which contain the same elongated core structure of a central  $\beta$  sheet and flanking helices. The structure seems to be relatively inflexible, as all E2 structures overlap closely and are unchanged in various protein complexes [9,10••–12••]. The catalytic cysteine that forms a thiolester with ubiquitin is located in a shallow depression on one face of the E2 surface.

The known structures include E2 complexes with large fragments of the HECT domain E3 E6-AP (Figure 2a) [9] and with the RING domain E3 c-Cbl (Figure 2b) [10••]. Both of these E3 structures are complexed with the same E2, UbcH7, which, remarkably, binds the two very different HECT and RING structures in a large part through the same two loops at one end of the E2 structure (Figure 3). The prominent role of UbcH7 Phe63 in binding the E3s suggests that the identity of this residue correlates with cognate E2–E3 pairs [9,13]. Indeed, UbcH7 Phe63 contacts both Ile383 and Trp408 of the c-Cbl RING domain, and all three of these residues co-vary with different cognate E2–RING E3 complexes [10••]. For both the HECT and RING complexes, E2 binds in a relatively hydrophobic groove on the E3. The presence or absence of this groove on the numerous RING domains may correlate with RING proteins that function as E3s versus those that perform other biological functions [10••].

Sequence comparisons suggest that the RING domain family includes very distant relatives called U-box proteins [14•]. Remarkably, these apparently similar folds are so diverged that the coordinated zinc ions that stabilize

Figure 1



Cartoons of the ubiquitin system and E3 architectures. (a) The ubiquitin system of post-translational modification. Ubiquitin (U) is activated by an E1 enzyme, transferred to an E2 enzyme and ligated to substrate by an E2-E3 complex. (b) HECT domain E3 E6-AP-UbcH7 structure. (c) c-Cbl-UbcH7 structure. RING and substrate-binding domains are contained within a single polypeptide. (d) SCF or VCB structures. The substrate-binding and adaptor-binding domains of the F-box/SOCS-box subunit are shown as an oval and rectangle,

respectively. The N- and C-terminal domains of the cullin are shown as a cylinder and oval, respectively. A consistent color scheme is used for all figures throughout this review. Ubiquitin is in orange. E2 is shown in green with the catalytic cysteine shown as a yellow dot. The major E3 subunit/domain is blue. Substrate-binding subunit/domains are purple. Substrate is yellow. RING subunit or domain is red. The adaptor subunit is in pale cyan. Individual protein subunits have a black outline.

RING domains are replaced by residues that form buried salt bridges and hydrogen bonds in U-boxes. First identified as a domain within the Ufd2 protein, which is required for efficient polyubiquitylation of a model substrate [15], U-boxes have been shown to bind E2 enzymes and to autoubiquitylate when tested using the standard assay for RING E3 activity (reviewed in [16]). Like RING domains, U-boxes are typically embedded within longer proteins that also contain domains that probably function as protein-protein recognition motifs.

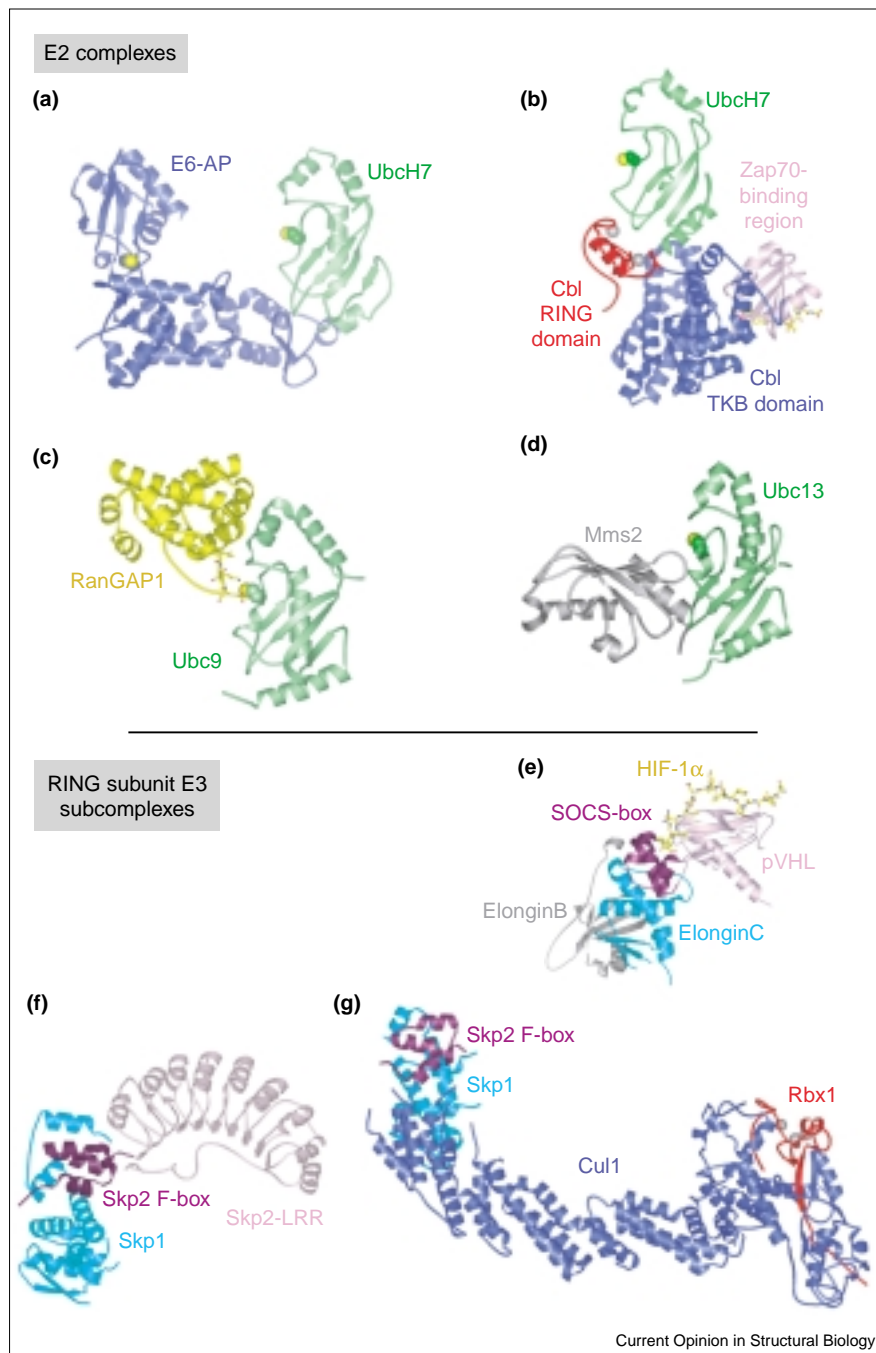
### Selection of substrate by E3s

Many different types of protein-protein recognition motif are used by E3s to select their substrates. For example, the HECT domain E3 Rsp5/Nedd4 binds substrates through its N-terminal WW domains [17,18]. The RING domain E3 MDM2 binds an amphiphilic helix of its p53 substrate in a deep hydrophobic cleft [19]. c-Cbl selects its substrates, which include activated cell surface receptors, by the binding of a phosphotyrosine residue to its variant SH2 domain [10<sup>••</sup>,20]. Substrate-binding domains of the numerous E3 F-box proteins often exhibit the well-known LRR (leucine-rich repeat) [21] or WD-40 [22] protein-protein interaction motifs, and typically bind substrates that have been selected for ubiquitylation by phosphorylation on serine or threonine sidechains [23]. Another post-translational

modification that leads to ubiquitylation is the recognition of *N*-glycosylation on proteins that are retrotranslocated from the endoplasmic reticulum into the cytosol [24]. The recent structure determination of the E3 Siah1a substrate-binding domain [25] revealed an intriguing similarity with the TRAF domain of TNF receptor-associated factors. This similarity seems unlikely to reflect similar modes of ligand binding, however, because the binding site in TRAF complexes is occluded in the Siah1a structure [25,26].

The recently elucidated interaction between the pVHL subunit of the E3 VCB and its substrate, the transcription factor HIF-1 $\alpha$ , provides an exquisite example of molecular recognition. HIF-1 $\alpha$  induces expression of genes that function in response to low oxygen tension (hypoxia). Normal oxygen levels repress the hypoxic response by inducing ubiquitylation and subsequent degradation of HIF-1 $\alpha$  via hydroxylation of a HIF-1 $\alpha$  proline using an oxygen-stimulated prolyl hydroxylase [27-29]. The hydroxyproline (Hyp) residue is recognized in the context of an extended polypeptide that positions the Hyp sidechain deep into a pocket of the pVHL  $\beta$  domain, where the hydrogen bonding requirement of the buried Hyp hydroxyl is satisfied by sidechains Ser111 and His115 of pVHL [30<sup>••</sup>,31<sup>••</sup>] (Figures 2e and 4). The pVHL structure does not change upon binding the Hyp peptide and

Figure 2



Gallery of some recently determined structures. Structures with bound E2 are shown above the line in the same relative orientation. RING subunit E3 complexes are shown below the line. Color scheme is the same as for Figure 1. (a) E2 UbCH7 complexed with the HECT domain E3 E6-AP [9]. (b) E2 UbCH7 complexed with the RING domain E3 c-Cbl [10<sup>••</sup>]. (c) E2 Ub9–RanGAP1 substrate structure [11<sup>••</sup>]. (d) E2 Ub13 forms a stable complex with the UEV protein Mms2 [12<sup>••</sup>,57<sup>•</sup>]. (e) Interaction between the pVHL subunit of VCB and its substrate HIF-1 $\alpha$  [30<sup>••</sup>,31<sup>••</sup>,32]. (f) Complex of Skp1 with the F-box protein Skp2 [37<sup>•</sup>]. (g) Core SCF complex comprising Skp1, the F-box fragment of Skp2, Cul1 and Rbx1 [38<sup>••</sup>].

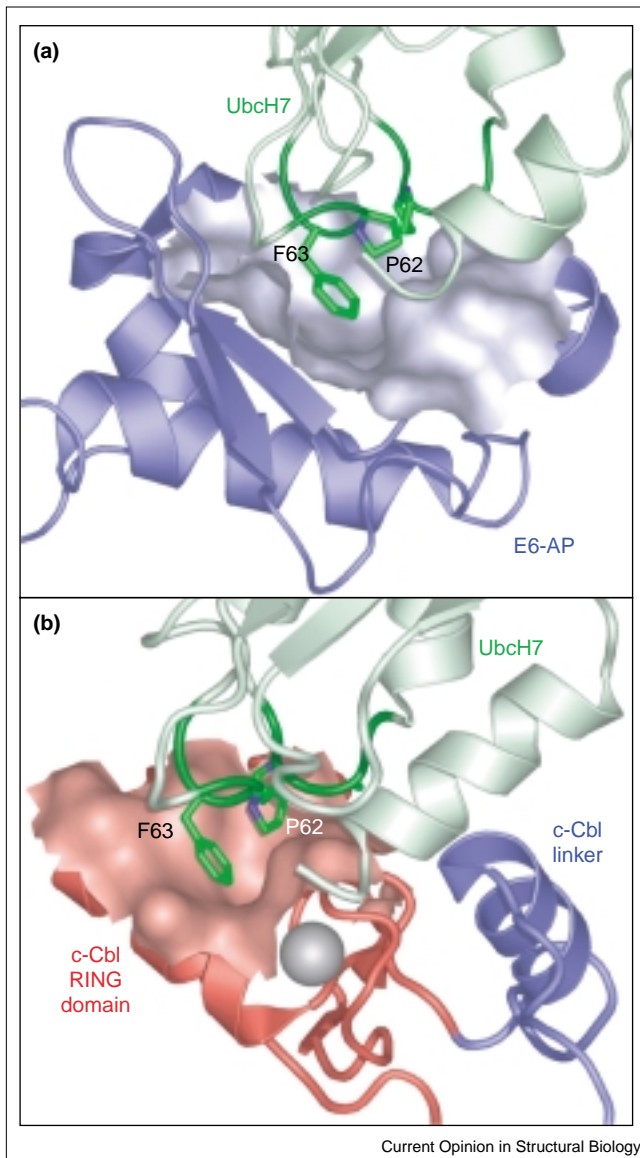
the position of the Hyp hydroxyl is occupied by an ordered water molecule in the apo structure [32]. These findings explain how hydroxylated HIF-1 $\alpha$  is selected by pVHL, as the constrained hydrogen bonding capacity of the specificity pocket excludes the unmodified proline residue.

### Interactions of substrate-binding and adaptor subunits of RING subunit E3s

The RING subunit E3s SCF and VCB each recognize substrate-binding subunits by means of their respective adaptor subunits, Skp1 and elonginCB. Skp1 binds to the

F-box [33] and elonginCB binds to the SOCS-box [34–36] domains of proteins that also possess a variable substrate-binding domain. The Skp1–F-box interaction has been revealed in two crystal structures, one a complex of Skp1 with the F-box protein Skp2 [37<sup>•</sup>] (Figure 2f), the other is a core SCF complex comprising Skp1, the F-box fragment of Skp2, Cul1 (cullin) and Rbx1 (the RING subunit) [38<sup>••</sup>] (Figure 2g). The F-box is a three-helix bundle that packs against Skp1 through two interfaces (core and variable) to bury  $\sim 3000 \text{ \AA}^2$  of mostly hydrophobic surface (Figure 5a). The core interface accounts for approximately two-thirds

Figure 3

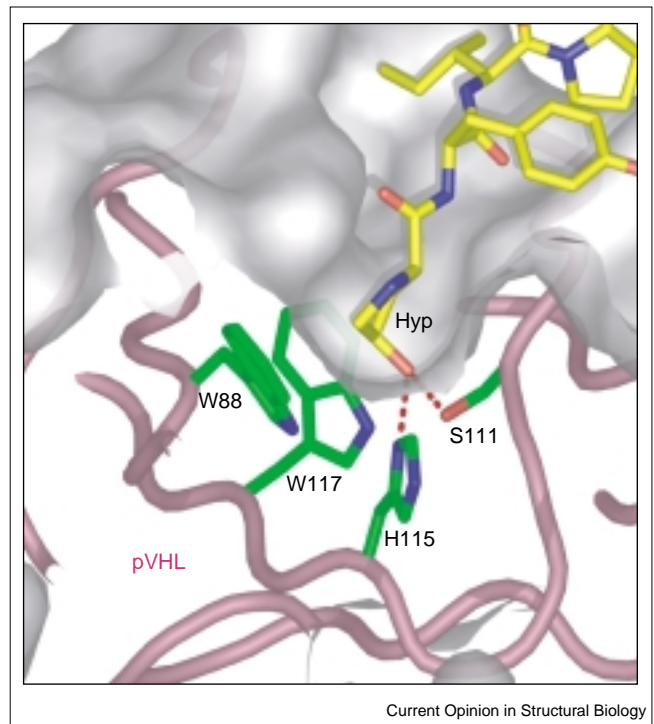


HECT-E2 (a) and RING-E2 (b) interfaces. In addition to the RING domain, c-Cbl also contacts E2 through its ordered 'linker sequence' (blue).

of the buried surface area and contains residues conserved in all Skp1 and F-box protein family members. The opposite face of the F-box packs against the C-terminal helix of Skp1 to form the variable interface, which is composed of residues that are conserved among Skp1 orthologs, but not between other Skp1 family members or between different F-box proteins. This structure and the observation that the C-terminal helix of Skp1 makes a significant contribution to binding affinity [37<sup>\*</sup>] suggest that the different Skp1 orthologs probably function in partnership with different subsets of F-box proteins.

SOCS-box proteins bind to the adaptor protein elonginC, which exists in a stable complex with the protein elonginB.

Figure 4



pVHL mainchain (pink) with selected sidechains (green) and molecular surface (gray). The bound HIF-1 $\alpha$  peptide is yellow and the hydrogen bonds are red.

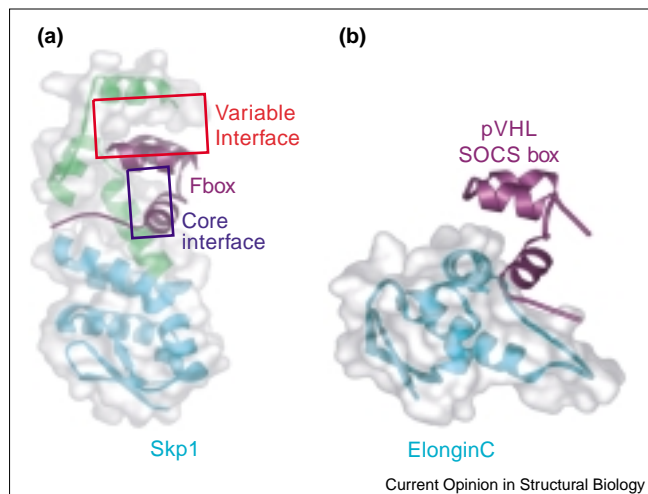
Consistent with the possibility of evolutionary relationships suggested from sequence similarity, the SOCS-box adopts a similar three-helix structure to the F-box, and elonginC and the N-terminal portion of Skp1 share similar structures [30<sup>\*\*</sup>]. This relationship is highly diverged, however, as the organization of the SOCS-box-elonginC and F-box-Skp1 interfaces are quite different, with the contacts mediated by nonequivalent parts of Skp1 and elonginC (Figure 5).

### Scaffolding and conformational changes

A striking feature of the known E3 structures is that the substrate-recognition site is always distant from the site of ubiquitylation. For example, the phosphotyrosine-binding site of c-Cbl is 60 Å distant from the active site cysteine of the bound E2 enzyme [10<sup>\*\*</sup>]. It is not known how many residues separate the phosphotyrosine from the ubiquitinated lysine(s) of c-Cbl substrates, although the compact structure argues against significant conformational changes and suggests a separation of at least 20 residues.

The SCF scaffolding protein, Cul1, has an elongated 415-residue N-terminal domain composed of three repeats of a novel five-helix structure and a globular 360-residue C-terminal domain [38<sup>\*\*</sup>] (Figure 2g). Skp1 binds at the N-terminal tip of Cul1 through an interface whose conservation among cullin orthologues presumably reflects the specific pairing of different cullins with different Skp1 family members, including elonginC. The Skp1-Cul1

Figure 5



Interactions between adaptor and substrate-binding subunits in RING subunit E3s. (a) F-box–Skp1 interface. The N-terminal domain of Skp1, which is structurally similar to elonginC, is shown in cyan. The C-terminal domain of Skp1, which contacts the F-box and does not have a counterpart in elonginC, is displayed in green. (b) SOCS-box–elonginC interface. The F-box and SOCS-box are in equivalent orientations. As shown, this does not correspond to similar orientations of elonginC and the Skp1 N-terminal domain.

interface extends to include Skp2–Cul1 contacts, which are mediated in part by conserved F-box residues. At the opposite end of the extended cullin structure, the Cul1 C-terminal domain binds the RING subunit, Rbx1, by insertion of an Rbx1  $\beta$  strand into a sheet that is otherwise comprised of strands from Cul1. Sequence conservation suggests that the equivalent interface is formed between other cullin–RING pairs [38\*\*].

The SCF core structure suggests a rigidly defined architecture that does not possess significant flexibility in the relative orientation of the various domains or subunits. This impression is reinforced by the lack of activity in an SCF complex constructed with a variant Cul1 that was designed to have a flexible link between the Cul1 N- and C-terminal domains [38\*\*]. Assuming both a lack of flexibility and that the E2–RING interaction of SCF is the same as for c-Cbl, it appears that the E2 active site is  $\sim 50$  Å from Skp2 [38\*\*]. This is consistent with the at least 22-residue separation seen between the phosphothreonine of the p27 substrate that is known to bind Skp2 and the probable ubiquitylation sites [39].

Although the currently available RING E3 structures do not suggest conformational change, a requirement for substantial movement is implied by the crystal structure of the HECT domain E3 E6-AP (Figure 2a). Despite the direct transfer of the ubiquitin C terminus from the E2 to the HECT domain cysteine, the E2 and HECT E3 active site thiols are 41 Å apart [9]. (Cysteine residues are shown as yellow spheres in Figure 2a.) It is currently unclear how

this distance is bridged, but significant rearrangement must be required.

### Other subunits and interactions

The possibility that the SCF and VCB E3 complexes might contain additional subunits is suggested by comparison with APC (anaphase promoting complex; cyclosome), another RING subunit E3 that has only been visualized at low resolution [40]. Like SCF and VCB, APC appears to be built upon a cullin-like scaffolding protein, although many more subunits (11 human, 12 yeast) have been identified. Thus, it is possible that the extra APC subunits have less tightly associated counterparts in SCF and VCB. Direct evidence has been reported for additional SCF subunits required to bind certain substrates [41–44] and possibly to recruit the SCF core complex to kinetochores [45] and to the RAR1 signaling protein [46].

Structures have been determined of the Doc domain of the APC10 subunit from human [47] and yeast [48] APC. The Doc domain adopts a ‘jelly roll’ fold, which has been seen in many other protein structures and typically functions to bind a ligand, such as a sugar, nucleotide, phospholipid, nucleic acid or protein. Interestingly, the conserved APC10 residues cluster at the putative ligand-binding cleft and a temperature-sensitive mutation maps to this site [49]. The occurrence of Doc domains in several HECT and RING E3s suggest that they play an important role in the ubiquitylation reaction [50,51], although what that role might be and the identity of the relevant Doc domain ligands are currently unknown.

Some APC substrates are targeted by their interaction with the Cdc20 protein, a process that is inhibited by the binding of the checkpoint protein Mad2 to Cdc20. Recent structure determinations have revealed a dramatic conformational change in Mad2 upon binding to a peptide derived from the binding motif of Cdc20 [52,53]. This rearrangement involves displacement of two  $\beta$  strands from one edge of a  $\beta$  sheet to the other edge, with wrapping of the Mad2 polypeptide chain around the bound peptide. The same ‘seat-belt’ structure is formed in a complex of Mad2 with Mad1, which binds to the same site on Mad2 to regulate the Mad2–Cdc20 interaction [54].

The E2 Ubc13 forms a stable complex with the ubiquitin E2 variant (UEV) protein Mms2 (Figure 2d). UEV domains share structural and sequence similarity with authentic E2s, but lack the cysteine residue that is essential for catalytic activity, as well as the two C-terminal helices. Mms2 performs an E3-like role because the Ubc13–Mms2 complex catalyses the formation of polyubiquitin chains in which the Lys63 of one ubiquitin is linked to the C terminus of its neighbor [55,56]. Unlike Lys48-linked polyubiquitin, this type of chain performs biological roles that do not involve proteolysis [4]. The Ubc13–Mms2 crystal structure [12\*\*,57\*] revealed an asymmetric complex in which one end of Mms2 binds against a side of Ubc13 to create a cleft

**Figure 6 legend**

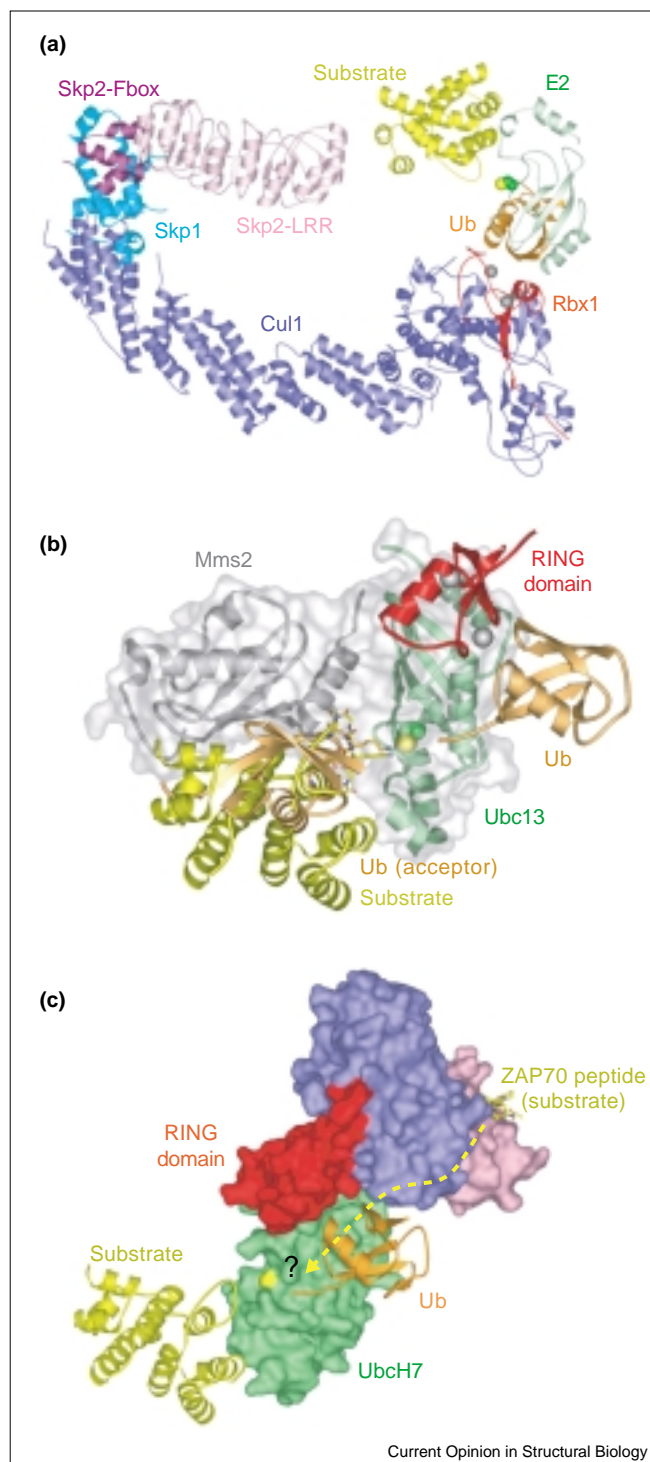
(Speculative models of E3–E2–substrate complexes with ubiquitin and substrate. The substrate models (yellow) were positioned by overlapping the Ubc9–RanGAP1 structure (Figure 2c) on the E2 subunit. Although the RanGAP1 domain is not known to be a substrate for the ligase complexes shown here, its full structure is shown to convey a sense of scale. The thioester-bound ubiquitin (Ub) was positioned on the E2 structure as described [65,66]. (a) SCF. This model was constructed by overlapping equivalent parts of the Skp1–Skp2 (Figure 2f) and core SCF complex (Figure 2g) structures. The E2 was positioned by overlap of the RING subunit with the c-Cbl structure (Figure 2b). In these models, the distance to the E2 cysteine residue is ~50 Å from the nearest atom of Skp2, consistent with the known sites of substrate phosphorylation and ubiquitylation. Figure 2e shows the VCB–substrate complex oriented after overlap of elonginC on the equivalent part of Skp1 shown in this model. Substitution of the VCB structure into the SCF model would position the substrate Hyp residue ~75 Å from the E2 cysteine. (b) Mms2–Ubc13. E3 RING domain and acceptor (substrate) and donor (thioester-bound) ubiquitins were modeled as described in [12••]. The close overlap seen between RanGAP1 substrate and the ubiquitin substrate support the suggestion that ubiquitin–E2 interactions mimic those of RanGAP1–Ubc9. (c) c-Cbl. The dotted yellow arrow indicates a groove on the c-Cbl surface proposed to bind the extended substrate between the sites of phosphorylation and ubiquitylation [10••]. This path appears incompatible with the modeled ubiquitin. Panels (b,c) are rotated 180° about the horizontal axis compared to the E2 orientations in panel (a) and Figure 2.

that appears appropriate to bind ubiquitin in an orientation to form Lys63-linked chains.

The one other UEV domain of known structure is from the Tsg101 protein, which functions in the vacuolar sorting pathway [58], and in budding of HIV and Ebola virus particles [59–61]. This structure was determined alone [62•] and in complex with a peptide containing the viral Gag protein PTAP sequence [63]. It is not yet known, however, if the Tsg101 UEV binds an E2, like Mms2–Ubc13, or if it participates in an E3 complex.

**E2 interactions with ubiquitin and substrate**

It seems likely that E2 enzymes only make minimal Pcontacts with substrate proteins, because conserved sequence motifs around ubiquitinated lysines have not been identified. In contrast, a tetrapeptide ( $\Psi$ -K-x-D/E;  $\Psi$ , hydrophobic; K, SUMOated lysine) sequence motif has been established for the E2 enzyme (Ubc9) that catalyzes conjugation to the ubiquitin-like protein SUMO [64]. Recently, the crystal structure of Ubc9 was determined in complex with a large fragment of the SUMOylation substrate RanGAP1 [11••] (Figure 2c). Ubc9 makes specific interactions with the consensus motif that positions the substrate lysine N $\zeta$  atom 3.5 Å from the catalytic cysteine of the E2. As expected, the substrate lysine approaches the E2 cysteine from the opposite direction to the thioester-bound SUMO/ubiquitin, which has been mapped by analysis of chemical shift changes upon E2–ubiquitin complex formation [65,66]. One unresolved aspect of ubiquitylation/SUMOylation is how nucleophilic attack of the lysine on the thiol-bound ubiquitin might be promoted by groups on E2. The emerging view is that transfer occurs

**Figure 6**

because the E2–ubiquitin thioester bond is naturally labile and the E2–E3 complex appropriately positions substrate lysine, without need for further activation [11••].

The close similarity between biochemical pathways of ubiquitylation and SUMOylation suggests that ubiquitylation might involve similar interactions to those seen for Ubc9–RanGAP1, albeit in a way that is relatively insensitive

to the identity of amino acid residues that contact E2. We have developed this idea by overlapping the Ubc9–RanGAP1 structure on the E2 subunit of E2–E3 complexes. For SCF, this positions the substrate, as expected, toward the substrate-binding domain of the F-box protein (Figure 6a). Similarly, for Ubc13–Mms2, overlap on the E2 subunit positions RanGAP1 in the cleft that modeling and mutagenesis suggest binds the acceptor (substrate) ubiquitin (Figure 6b). The equivalent modeling exercise for c-Cbl (Figure 6c) suggests that the substrate-binding groove proposed by Zheng *et al.* [10••] is blocked by the thiolester-bound ubiquitin. This suggests that the substrate may traverse an alternative path between the primary substrate-binding site and the site of ubiquitylation, although the location of the thiolester-bound ubiquitin has not been rigorously defined for this complex. These models show that the Ubc9–RanGAP1 interaction may mimic that of ubiquitylation substrates with other E2 enzymes, although confirmation of this speculation will require experimental verification.

## Conclusions

Recent years have seen several landmark structure determinations that have greatly advanced understanding of how E3 complexes are assembled. Despite the considerable progress, however, many important structural questions remain to be answered, including questions of substrate recognition, conformational changes, E3 activation/regulation and higher order assembly. Some alternative larger E3 architectures have already been indicated, such as the APC E3 complex and a variant SCF-like complex that assembles on a scaffold of the adenomatous polyposis coli protein, and tethers Siah1 and Skp1 via the SIP protein [67,68]. Future structural studies are needed to further illuminate the extraordinary complexity, biochemical mechanisms and biological significance of the ubiquitylation machinery.

## Acknowledgements

This work was supported by National Institutes of Health grants RO1 GM59135 and T32 CA93247. We thank Marty Rechsteiner, Wes Sundquist, and members of the Hill and Sundquist laboratories for critical comments on the manuscript.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- Hershko A, Ciechanover A: **The ubiquitin system.** *Annu Rev Biochem* 1998, **67**:425-479.
2. Hochstrasser M: **All in the ubiquitin family.** *Science* 2000, **289**:563-564.
  3. Pickart CM: **Mechanisms underlying ubiquitination.** *Annu Rev Biochem* 2001, **70**:505-533.
  4. Pickart CM: **Ubiquitin in chains.** *Trends Biochem Sci* 2000, **25**:544-548.
  5. Scheffner M, Nuber U, Huibregtse JM: **Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thiolester cascade.** *Nature* 1995, **373**:81-83.
  6. Joazeiro CA, Weissman AM: **RING finger proteins: mediators of ubiquitin ligase activity.** *Cell* 2000, **102**:549-552.
  7. Kamura T, Koepp DM, Conrad MN, Skowrya D, Moreland RJ, Iliopoulos O, Lane WS, Kaelin WJ, Elledge SJ, Conaway RC *et al.*: **Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase.** *Science* 1999, **284**:657-661.
  8. Skowrya D, Koepp DM, Kamura T, Conrad MN, Conaway RC, Conaway JW, Elledge SJ, Harper JW: **Reconstitution of G1 cyclin ubiquitination with complexes containing SCFGrr1 and Rbx1.** *Science* 1999, **284**:662-665.
  9. Huang L, Kinnucan E, Wang G, Beaudenon S, Howley PM, Huibregtse JM, Pavletich NP: **Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade.** *Science* 1999, **286**:1321-1326.
  10. Zheng N, Wang P, Jeffrey PD, Pavletich NP: **Structure of a c-Cbl-•• UbcH7 complex: RING domain function in ubiquitin-protein ligases.** *Cell* 2000, **102**:533-539.
- The primary substrate-binding site is seen to be 60 Å from the E2 catalytic cysteine, although the compact structure argues against a conformational change. The E2 binds c-Cbl through the same surface it uses to bind E6-AP, although the c-Cbl and E6-AP structures are quite different.
11. Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD: **Structural •• basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin conjugating enzyme Ubc9 and RanGAP1.** *Cell* 2002, **108**:345-356.
- The structural basis is revealed for the recognition of SUMOylation substrates by the E2 Ubc9. The absence of titratable groups near the active site suggests that catalysis proceeds without the assistance of a base to enhance nucleophilicity of the lysine residue.
12. VanDemark AP, Hofmann RM, Tsui C, Pickart CM, Wolberger C: **•• Molecular insights into polyubiquitin chain assembly: crystal structure of the Mms2/Ubc13 heterodimer.** *Cell* 2001, **105**:711-720.
- This report, together with [57\*], shows that a combination of structure determination and mutagenesis/biochemistry suggests that the acceptor ubiquitin is oriented for conjugation on Lys63 by binding in a channel formed at the interface of Ubc13 and Mms2.
13. Nuber U, Scheffner M: **Identification of determinants in E2 ubiquitin-conjugating enzymes required for hect E3 ubiquitin-protein ligase interaction.** *J Biol Chem* 1999, **274**:7576-7582.
  14. Aravind L, Koonin EV: **The U box is a modified RING finger - a • common domain in ubiquitination.** *Curr Biol* 2000, **10**:R132-134.
- Sequence comparisons and modeling indicate a common fold for RING finger and U-box domains, despite replacement of RING zinc ion ligands with polar sidechains.
15. Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU, Jentsch S: **A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly.** *Cell* 1999, **96**:635-644.
  16. Cyr DM, Hohfeld J, Patterson C: **Protein quality control: U-box-containing E3 ubiquitin ligases join the fold.** *Trends Biochem Sci* 2002, **27**:368-375.
  17. Wang G, Yang J, Huibregtse JM: **Functional domains of the Rsp5 ubiquitin-protein ligase.** *Mol Cell Biol* 1999, **19**:342-352.
  18. Kanelis V, Rotin D, Forman-Kay JD: **Solution structure of a Nedd4 WW domain-ENaC peptide complex.** *Nat Struct Biol* 2001, **8**:407-412.
  19. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, Pavletich NP: **Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain.** *Science* 1996, **274**:948-953.
  20. Meng W, Sawasdikosol S, Burakoff SJ, Eck MJ: **Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase.** *Nature* 1999, **398**:84-90.
  21. Kobe B, Kajava AV: **The leucine-rich repeat as a protein recognition motif.** *Curr Opin Struct Biol* 2001, **11**:725-732.
  22. Smith TF, Gaitatzes C, Saxena K, Neer EJ: **The WD repeat: a common architecture for diverse functions.** *Trends Biochem Sci* 1999, **24**:181-185.
  23. Jackson PK, Eldridge AG, Freed E, Furstenthal L, Hsu JY, Kaiser BK, Reimann JD: **The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases.** *Trends Cell Biol* 2000, **10**:429-439.
  24. Yoshida Y, Chiba T, Tokunaga F, Kawasaki H, Iwai K, Suzuki T, Ito Y, Matsuoka K, Yoshida M, Tanaka K *et al.*: **E3 ubiquitin ligase that recognizes sugar chains.** *Nature* 2002, **418**:438-442.

25. Polekhina G, House CM, Traficante N, Mackay JP, Relaix F, Sassoon DA, Parker MW, Bowtell DD: **Siah ubiquitin ligase is structurally related to TRAF and modulates TNF-alpha signaling.** *Nat Struct Biol* 2002, **9**:68-75.
26. Reed JC, Ely KR: **Degrading liaisons: Siah structure revealed.** *Nat Struct Biol* 2002, **9**:8-10.
27. Epstein ACR, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzén E, Wilson MI, Dhanda A *et al.*: ***C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation.** *Cell* 2001, **107**:43-54.
28. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WGJ: **HIF-1 $\alpha$  targeted for VHL-mediated destruction by proline hydroxylation: implications for O<sub>2</sub> sensing.** *Science* 2001, **292**:464-468.
29. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim A, Hestrestreit HF, Mukherji M, Schofield CJ *et al.*: **Targeting of HIF-1 $\alpha$  to the von Hippel-Lindau ubiquitylation complex by O<sub>2</sub>-regulated prolyl hydroxylation.** *Science* 2001, **292**:468-472.
30. Min JH, Yang H, Ivan M, Gertler F, Kaelin WGJ, Pavletich NP: **Structure of an HIF-1 $\alpha$ -pVHL complex: hydroxyproline recognition in signaling.** *Science* 2002, **296**:1886-1889.
- This report, together with [31\*\*], shows that pVHL specifically binds a Hyp residue of its HIF-1 $\alpha$  substrate through two hydrogen bonds at the bottom of a hydrophobic pocket. Surface plasmon resonance confirmed the role of individual residues in binding suggested by the crystal structure.
31. Hon WC, Wilson MI, Harlos K, Claridge TD, Schofield CJ, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DI, Jones EY: **Structural basis for the recognition of hydroxyproline in HIF-1 $\alpha$  by pVHL.** *Nature* 2002, **417**:975-978.
- See annotation to [30\*\*].
32. Stebbins CE, Kaelin WGJ, Pavletich NP: **Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function.** *Science* 1999, **284**:455-461.
33. Kipreos ET, Pagano M: **The F-box protein family.** *Genome Biol* 2000, **1**:3002.3001-3002.3007.
34. Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ: **The SOCS box: a tale of destruction and degradation.** *Trends Biochem Sci* 2002, **27**:235-241.
35. Alexander WS: **Suppressors of cytokine signalling (SOCS) in the immune system.** *Nat Rev Immunol* 2002, **2**:410-416.
36. Kamura T, Sato S, Haque D, Liu L, Kaelin WGJ, Conaway RC, Conaway JW: **The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families.** *Genes Dev* 1998, **15**:3872-3881.
37. Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinnucan ER, Finnin MS, Elledge SJ, Harper JW, Pavletich NP: **Insights into SCF ubiquitin ligases from the structure of the Skp1-Skp2 complex.** *Nature* 2000, **408**:381-386.
- The Skp2 F-box binds Skp1 through core and variable interfaces. The F-box and substrate-binding domain of Skp2 are packed against each other, rather than being connected through a flexible tether.
38. Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepf DM, Elledge SJ, Pagano M *et al.*: **Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex.** *Nature* 2002, **416**:703-709.
- The cullin Cul1 is shown to have an elongated structure that binds Skp1-F-box-substrate at its N-terminal end and the RING subunit at its C-terminal end. A model is proposed for how the substrate is presented to the E2.
39. Shirane M, Harumiya Y, Ishida N, Hirai A, Miyamoto C, Hatakeyama S, Nakayama K, Kitagawa M: **Down-regulation of p27(Kip1) by two mechanisms, ubiquitin-mediated degradation and proteolytic processing.** *J Biol Chem* 1999, **274**:13886-13893.
40. Gieffers C, Dube P, Harris JR, Stark H, Peters JM: **Three-dimensional structure of the anaphase-promoting complex.** *Mol Cell* 2001, **7**:907-913.
41. Margottin F, Bour SP, Durand H, Selig L, Benichou S, Richard V, Thomas D, Strebél K, Benarous R: **A novel human WD protein, h- $\beta$ TrCp, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif.** *Mol Cell* 1998, **1**:565-574.
42. Stone DM, Murone M, Luoh S, Ye W, Armanini MP, Gurney A, Phillips H, Brush J, Goddard A, de Sauvage FJ *et al.*: **Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli.** *J Cell Sci* 1999, **112**:4437-4448.
43. Ganoth D, Bornstein G, Ko TK, Larsen B, Tyers M, Pagano M, Herskko A: **The cell-cycle regulatory protein Cks1 is required for SCF(Skp2)-mediated ubiquitylation of p27.** *Nat Cell Biol* 2001, **3**:321-324.
44. Spruck C, Strohmaier H, Watson M, Smith AP, Ryan A, Krek TW, Reed SI: **A CDK-independent function of mammalian Cks1: targeting of SCF(Skp2) to the CDK inhibitor p27Kip1.** *Mol Cell* 2001, **7**:639-650.
45. Kitagawa K, Skowrya D, Elledge SJ, Harper JW, Hieter P: **SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex.** *Mol Cell* 1999, **4**:21-33.
46. Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P: **The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance.** *Science* 2002, **295**:2073-2076.
47. Wendt KS, Vodermaier HC, Jacob U, Gieffers C, Gmachl M, Peters JM, Huber R, Sondermann P: **Crystal structure of the APC10/DOC1 subunit of the human anaphase-promoting complex.** *Nat Struct Biol* 2001, **8**:784-788.
48. Au SW, Leng X, Harper JW, Barford D: **Implications for the ubiquitination reaction of the anaphase-promoting complex from the crystal structure of the Doc1/Apc10 subunit.** *J Mol Biol* 2002, **316**:955-968.
49. Hwang LH, Murray AW: **A novel yeast screen for mitotic arrest mutants identifies DOC1, a new gene involved in cyclin proteolysis.** *Mol Biol Cell* 1997, **8**:1877-1887.
50. Kominami K, Seth-Smith H, Toda T: **Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast.** *EMBO J* 1998, **17**:5388-5399.
51. Grossberger R, Gieffers C, Zachariae W, Podtelejnikov AV, Schleiffer A, Nasmyth K, Mann M, Peters JM: **Characterization of the DOC1/APC10 subunit of the yeast and the human anaphase-promoting complex.** *J Biol Chem* 1999, **274**:14500-14507.
52. Luo X, Fang G, Coldiron M, Lin Y, Yu H, Kirschner MW, Wagner G: **Structure of the Mad2 spindle assembly checkpoint protein and its interaction with Cdc20.** *Nat Struct Biol* 2000, **7**:224-229.
53. Luo X, Tang Z, Rizo J, Yu H: **The Mad2 spindle checkpoint protein undergoes similar major conformational changes upon binding to either Mad1 or Cdc20.** *Mol Cell* 2002, **9**:59-71.
54. Sironi L, Mapelli M, Knapp S, De Antoni A, Jeang KT, Musacchio A: **Crystal structure of the tetrameric Mad1-Mad2 core complex: implications of a 'safety belt' binding mechanism for the spindle checkpoint.** *EMBO J* 2002, **21**:2496-2506.
55. Hofmann RM, Pickart CM: **Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair.** *Cell* 1999, **96**:645-653.
56. Hoegge C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S: **RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO.** *Nature* 2002, **419**:135-141.
57. Moraes TF, Edwards RA, McKenna S, Pastushok L, Xiao W, Glover JN, Ellison MJ: **Crystal structure of the human ubiquitin conjugating enzyme complex, hMms2-hUbc13.** *Nat Struct Biol* 2001, **8**:669-673.
- See annotation to [12\*\*].
58. Katzmán DJ, Babst M, Emr SD: **Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I.** *Cell* 2001, **106**:145-155.
59. Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Cote M, Rich RL *et al.*: **Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding.** *Cell* 2001, **107**:55-65.
60. Martin-Serrano J, Zang T, Bieniasz PD: **HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress.** *Nat Med* 2001, **7**:1313-1319.



61. VerPlank L, Bouamr F, LaGrassa TJ, Agresta B, Kikonyogo A, Leis J, Carter CA: **Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag).** *Proc Natl Acad Sci USA* 2001, **98**:7724-7729.
62. Pornillos O, Alam SL, Rich RL, Myszka DG, Davis DR, Sundquist WI:  
• **Structure and functional interactions of the Tsg101 UEV domain.** *EMBO J* 2002, **21**:2397-2406.  
Compared to canonical E2s, the Tsg101 UEV has an extended  $\beta$  hairpin that links strands 1 and 2, and lacks the two C-terminal helices. PTAP-containing peptides bind in a hydrophobic cleft exposed by the absence of the C-terminal helices and ubiquitin binds in a novel site surrounding the  $\beta$  hairpin.
63. Pornillos O, Alam SL, Davis DR, Sundquist WI: **Structure of the Tsg101 UEV domain in complex with its peptide binding site in the HIV-1 p6 protein.** *Nat Struct Biol* 2002, **9**:812-817.
64. Melchior F: **SUMO–nonclassical ubiquitin.** *Annu Rev Cell Dev Biol* 2000, **16**:591-626.
65. Miura T, Klaus W, Gsell B, Miyamoto C, Senn H: **Characterization of the binding interface between ubiquitin and class I human ubiquitin-conjugating enzyme 2b by multidimensional heteronuclear NMR spectroscopy in solution.** *J Mol Biol* 1999, **290**:213-228.
66. Hamilton KS, Ellison MJ, Barber KR, Williams RS, Huzil JT, McKenna S, Ptak C, Glover M, Shaw GS: **Structure of a conjugating enzyme-ubiquitin thiolester intermediate reveals a novel role for the ubiquitin tail.** *Structure* 2001, **9**:897-904.
67. Matsuzawa SI, Reed JC: **Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses.** *Mol Cell* 2001, **7**:915-926.
68. Liu J, Stevens J, Rote CA, Yost HJ, Hu Y, Neufeld KL, White RL, Matsunami N: **Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein.** *Mol Cell* 2001, **7**:927-936.