The role of Elongin BC-containing ubiquitin ligases

Fumihiko Okumura*, Mariko Matsuzaki, Kunio Nakatsukasa and Takumi Kamura*

Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan

The Elongin complex was originally identified as a positive regulator of RNA polymerase II and is composed of a transcriptionally active subunit (A) and two regulatory subunits (B and C). The Elongin BC complex enhances the transcriptional activity of Elongin A. “Classical” SOCS box-containing proteins interact with the Elongin BC complex and have ubiquitin ligase activity. They also interact with the scaffold protein Cullin (Cul) and the RING domain protein Rbx and thereby are members of the Cullin RING ligase (CRL) superfamily. The Elongin BC complex acts as an adaptor connecting Cul and SOCS box proteins. Recently, it was demonstrated that classical SOCS box proteins can be further divided into two groups, Cul2- and Cul5-type proteins. The classical SOCS box-containing protein pVHL is now classified as a Cul2-type protein. The Elongin BC complex containing CRL family is now considered two distinct protein assemblies, which play an important role in regulating a variety of cellular processes such as tumorigenesis, signal transduction, cell motility, and differentiation.

Keywords: ubiquitin, Cullin, Elongin, ECS complex, SCF complex

INTRODUCTION
Polyubiquitin-mediated protein degradation plays an important role in the elimination of short-lived regulatory proteins (Peters, 1998), including those that contribute to the cell cycle, cellular signaling in response to environmental stress or extracellular ligands, morphogenesis, secretion, DNA repair, and organelle biogenesis (Hershko and Ciechanover, 1998). The system responsible for the attachment of ubiquitin to the target protein consists of several components that act in concert (Hershko and Ciechanover, 1992; Scheffner et al., 1995), including a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin–protein isopeptide ligase (E3). E3 is believed to be the component of the ubiquitin conjugation system that is most directly responsible for substrate recognition (Scheffner et al., 1995). Based on structural similarity, E3 enzymes have been classified into three families: the HECT (homologous to E6-AP COOH terminus) family (Huibregtse et al., 1995; Hershko and Ciechanover, 1998), the RING finger-containing protein family (Lorick et al., 1999; Freemont, 2000; Joazeiro and Weissman, 2000), and the U box family (Aravind and Koonin, 2000; Hatakeyama et al., 2001; Cyr et al., 2002). The 5 phase kinase-associated protein 1 (Skp1)–Cullin 1 (Cul1)–F box protein (SCF) family is a member of the RING finger-containing ubiquitin ligase family (Lipkowitz and Weissman, 2011). Cul1 is a scaffold protein and assembles multiple proteins into complexes, which include a small RING finger protein (Rbx1), an adaptor protein (Skp1), and a substrate-targeting protein (F box protein). Substrate recognition by the RING finger-containing ubiquitin ligase family is modulated by post-translational modifications of the target substrate, including phosphorylation, glycosylation, and sumoylation (Lipkowitz and Weissman, 2011). One substrate can be polyubiquitinated by different ubiquitin ligases and vice versa. The Elongin B and C–Cul2 or Cul5–SOCS box protein (ECS) family also belongs to the Cullin RING ligase (CRL) superfamily (Kile et al., 2002). SCF and ECS ubiquitin ligases have structural similarities in that both contain Rbx1 or Rbx2 as a RING finger protein and Cul1, Cul2, or Cul5 as a scaffold protein (Kile et al., 2002; Kamura et al., 2004). Although Skp1 is used as an adaptor protein in the SCF complex, the Elongin B and C complex is used as an adaptor in the ECS complex. Here, we review the Cul2- or Cul5-containing ECS ubiquitin ligase family, about which, compared to SCF ubiquitin ligases, relatively little is known.

THE ELONGIN COMPLEX
The Elongin complex is a positive regulator of RNA polymerase II (pol II) and increases the rate of elongation by suppressing transient pausing along the DNA template (Bradsher et al., 1993a,b). The Elongin complex is composed of a transcriptionally active A subunit and two regulatory subunits, B and C (Garrett et al., 1994, 1995; Aso et al., 1995). Elongin B and C form the Elongin BC complex, which enhances the transcriptional activity of Elongin A. Since Elongin B and C partially resemble ubiquitin and Skp1 (an adaptor of SCF-type ubiquitin ligases), respectively (Bai et al., 1996), they are able to serve as components of protein complexes with functions other than transcriptional regulation. For example, they were found to be components of the von Hippel–Lindau (VHL) tumor suppressor complex, which is also known as the ECS complex (Figure 1; Duan et al., 1995; Kibel et al., 1995).

Cul2-TYPE Ubiquitin Ligase
CRL2VHL Complex
von Hippel–Lindau disease is a hereditary cancer syndrome caused by germline mutations in the VHL tumor suppressor gene (Latif...
VHL disease and sporadic clear cell renal carcinomas are caused by mutation or deletion of the BC box, which reduces binding activity of the pVHL protein to its target proteins. Elongin BC–Cul2/5-containing ubiquitin ligases, such as the SCF and ECS-type ubiquitin ligases, play a crucial role in the degradation of the VHL protein and its target proteins. These ligases contain an F box protein, a Skp1 subunit, and a Cul1/Cul2 subunit, which together form a scaffold for ubiquitin ligase activity. The BC box of the VHL protein is recognized by the Cul2 box of the ubiquitin ligase complex, which is composed of Cul2, Rbx1, and Elongin BC subunits. The Cul2 box specifically recognizes the endogenous VHL box, which is composed of a BC box and a Cul2 box.

The pVHL protein contains the recently defined “VHL box,” which is composed of a BC box and a Cul2 box. The Cul2 box specifically recognizes the endogenous Cul2/Rbx1 complex in a similar manner to SCF-type ubiquitin ligase recognition by F box and Skp1. The Cul2 box determines the use of Cul2 as the scaffold protein. The ubiquitin ligase activity and targets the hypoxia-inducible factor-α (HIF-α) family of transcription factors (HIF-1–3α) for proteasomal degradation. As a result, HIF-α is polyubiquitinated and degraded. Three HIF prolyl hydroxylases (PHD1–3) have been identified in mammals and shown to hydroxylate HIF-α subunits. Since PHD2 is a critical enzyme for the hydroxylation of HIF-1α, PHD1, and 3 may hydroxylate other target substrates. In low oxygen conditions, PHDs are unable to hydroxylate the HIF-α subunits, which are therefore not recognized and targeted for degradation by pVHL. The unhydroxylated HIF-α dimerizes with constitutively expressed HIF-1β, which is implicated in the growth and progression of tumors.

The pVHL protein also polyubiquitinates and induces the degradation of Sprouty2 (Spry2), which is implicated in the growth and progression of tumors. Proline residues of Sprouty2 are hydroxylated by PHD at normoxia and are recognized by pVHL for polyubiquitination and degradation. Epidermal growth factor receptor (EGFR) is also targeted by pVHL for polyubiquitination and degradation.
FIGURE 2 | Regulation of HIF-α protein by pVHL. In normoxia, two proline residues of HIF-α are hydroxylated by PHD1–3, and then HIF-α is recognized by pVHL for polyubiquitination and degradation by the proteasome. On the other hand, in hypoxia, HIF-α escapes prolyl hydroxylation and thereby escapes degradation. HIF-α then dimerizes with HIF-1β to form an active transcription complex. Prolonged activation of the HIF-α/β transcription complex is a major contributor to VHL disease.

Yang, 2011). pVHL is proposed to down-regulate tumor growth caused by prolonged signaling of activated EGFR (Zhou and Yang, 2011). pVHL also mediates the polyubiquitination of the atypical PKCs, PKCδ, and PKCζII (Okuda et al., 2001; Iturrioz and Parker, 2007). PKCζII interacts with Par6, which plays a critical role in the development of tight junction structures and apico-basal polarization. It also inhibits tight junction formation and thereby regulates polarity. PKCζ has a similar function, which is also inhibited by pVHL through ubiquitin-dependent degradation. pVHL also polyubiquitinates the seventh subunit of human RNA polymerase II (hsRBP7) and suppresses hsRBP7-dependent VEGF promoter transactivation, VEGF mRNA expression, and VEGF protein secretion (Na et al., 2003). The large subunit of RNA polymerase II (Rpb1), which has sequence and structural similarity to the pVHL-binding domain of HIF-1α, is bound and polyubiquitinated by pVHL (Figure 4; Kuznetsova et al., 2003). The interaction between pVHL and Rpb1 is enhanced by hyperphosphorylation of Rpb1 by UV radiation, which indicates that Rpb1 ubiquitination may have a role in transcription-coupled DNA repair (Figure 4; Svejstrup, 2002; Kuznetsova et al., 2003). Further study showed that proline 1465 of Rpb1, which is located within the LXXLAP motif, is hydroxylated mainly by PHD1 during oxidative stress (Mikhaylova et al., 2008). pVHL is necessary for the oxidative stress-dependent hydroxylation of Pro1465, the phosphorylation of Ser5, and the polyubiquitination of Rpb1 and its recruitment to the DNA (Mikhaylova et al., 2008). Surprisingly, in renal clear cell carcinoma (RCC), pVHL increased the protein abundance and non-degradative ubiquitination of Rpb1 (Mikhaylova et al., 2008). Polyubiquitination of Rpb1 in RCC cells by pVHL contributes to tumor growth by modulating gene expression (Mikhaylova et al., 2008). This is different from previous results found in PC12 cells, in which pVHL polyubiquitinates Rpb1 for protein degradation (Kuznetsova et al., 2003). How pVHL differentially regulates Rpb1 in cells of different origins awaits further investigation.

CRL2LRR-1 COMPLEX
Leucine-rich repeat protein (LRR)-1 contains a VHL box and physiologically interacts with the endogenous Cul2–Rbx1 complex (Figure 3; Kamura et al., 2004; Costessi et al., 2011). In fact, nematode LRR-1 degrades the Cip/Kip CDK-inhibitor CKI-1 in C. elegans to promote cell cycle progression in germ cells (Starostina et al., 2010). Human LRR-1 also polyubiquitinates the CDK-inhibitor p21Cip1; however, it does not affect cell cycle progression (Starostina et al., 2010). Rather, human LRR-1 targets cytoplasmic p21 for degradation to prevent the inhibition of the Rho/ROCK/LIMK pathway (Starostina et al., 2010). These data indicate that human LRR-1 is a negative regulator of cofilin, an actin-depolymerizing protein that decreases cell motility (Starostina et al., 2010).

CRL2FEM1B COMPLEX
Feminization-1 (FEM-1) also contains a VHL box and physiologically interacts with the endogenous Cul2–Rbx1 complex (Figure 3). Feminization-1 (FEM-1) contains a VHL box and physiologically interacts with the endogenous Cul2–Rbx1 complex (Figure 3; Jager et al., 2005). In C. elegans, FEM-1 interacts with CED-4, an Apaf-1 homolog, to promote apoptosis, suggesting an evolutionarily conserved role in apoptosis regulation (Chan et al., 2000). Nematode FEM-1 polyubiquitinates TRA-1, a Gli-family transcription factor and terminal effector of the sex determination pathway (Starostina et al., 2007). Human FEM-1 homolog B (FEM1B) interacts with and polyubiquitinates ankyrin repeat domain 37 (Ankrd37), which contains ankyrin repeats and a putative nuclear localization signal (NLS; Shi et al., 2011). Ankrd37 is highly enriched in mouse testis and is conserved from zebrafish to humans (Shi et al., 2011). These data indicate that the terminal step in sex determination is controlled by ubiquitin-mediated proteolysis.

Feminization-1 is polyubiquitinated by SEL-10, an F box and WD40 repeat protein, for proteasomal degradation (Jager et al., 2005).
et al., 2004). In mammalian cells, receptor for activated C kinase (RACK)1, also a WD40 repeat protein, associates with FEM1B and mediates the polyubiquitination and downregulation of FEM1B (Subauste et al., 2009). RACK1 also binds to the Elongin BC complex and promotes the ubiquitination of HIF-1α independently of the pVHL complex (Liu et al., 2007). Since the Elongin BC binding site in RACK1 is similar to that of pVHL, it has been suggested that RACK1 is a Cul2-type ubiquitin ligase (Liu et al., 2007).

**CRL2PRAME COMPLEX**

Preferentially expressed antigen of melanoma (PRAME) contains a VHL box and physiologically interacts with endogenous Cul2–Rbx1 complex (Kamura et al., 2004; Costessi et al., 2011). Genome-wide chromatin immunoprecipitation experiments revealed that PRAME is specifically enriched at enhancers and at transcriptionally active promoters that are also bound by nuclear transcription factor Y (NFY), a transcription factor essential for early embryonic development (Bhattacharya et al., 2003; Costessi et al., 2011). However, the physiological substrates of PRAME have not yet been identified.

**Cul5-TYPE UBIQUITIN LIGASE**

**CRL5Cis/SOCS COMPLEX**

This family consists of suppressor of cytokine signaling (SOCS) proteins and cytokine-inducible Src homology 2 (SH2) domain-containing protein (CIS, also known as CISH), which
also interacts with the Elongin BC complex through its SOCS box (Piessevaux et al., 2008). To date, eight CIS/SOCS family proteins have been identified: CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, and SOCS7. All of them have a central SH2 domain as well as a C-terminally located SOCS box consisting of a 40-amino acid motif (Figure 5; Endo et al., 1997; Naka et al., 1997; Starr et al., 1997). Members of the CIS/SOCS family bind to janus kinases (JAKs), certain cytokine receptors, or signaling molecules, thereby suppressing downstream signaling events (Piessevaux et al., 2008). A small kinase inhibitory region (KIR) of SOCS1 and SOCS3 inhibits the JAKs by acting as a pseudo-substrate, resulting in the downregulation of further signal transduction (Piessevaux et al., 2008). The CIS/SOCS family can also down-regulate signaling by competing with downstream molecules for binding to the activated receptors (Ram and Waxman, 1999; Piessevaux et al., 2008) and can prevent signaling by polyubiquitination and degradation of target substrates. For example, SOCS1 polyubiquitinates JAK2, Vav, IRS1, and IRS2 (De Sepulveda et al., 2000; Kamizono et al., 2001, 2004; Babon et al., 2009; Sartori da Silva et al., 2009). Recent studies also demonstrated that SOCS1 and SOCS3 are important regulators of adaptive immunity (Kile et al., 2002; Tamiya et al., 2011). Some SOCS box-containing proteins – for example, CIS, SOCS1–7, SPRY domain-containing SOCS box proteins (SSB1, SSB2, and SSB4, also known as SPSB1, 2, and 4, respectively), ras-related protein Rab-40C (also known as RAR3), WD40 repeat-containing SOCS box protein WSB1, leucine-rich repeat protein MUF1, and ankyrin repeat- and SOCS box-containing protein (ASB)11 – also contain a BC box and a Cul5 box inside the SOCS box (Figure 5; Hilton et al., 1998; Kamura et al., 2001, 2004; Babon et al., 2009; Sartori da Silva et al., 2010). The amino acid sequence LPΦP in the Cul5 box results in a specific interaction with Cul5, particularly when there is a proline in the fourth position of the motif (Kamura et al., 2004). Furthermore, endogenous Cul5 interacts with endogenous Rbx2, enabling SOCS box-containing proteins to form a protein complex with Cul5 and Rbx2 (Figure 1C; Kamura et al., 1999, 2004; Ohta et al., 1999). The selective interactions between Cul2 and Rbx1 or Cul5 and Rbx2 suggest that Rbx1 and Rbx2 are functionally distinct, at least in terms of their specific binding to Cullin family members. Although SOCS1 contains a Cul5 box, no interaction between SOCS1 and Cul5 has been detected, most likely because the Cul5 box is incompletely conserved (Kamura et al., 2004). Since SOCS1 polyubiquitinates JAK2, Vav, IRS1, and IRS2 (De Sepulveda et al., 2000; Kamizono et al., 2001, 2004; Rui et al., 2002), it is possible that the interaction of SOCS1 with these substrates recruits other ubiquitin ligase(s) that actually mediate their polyubiquitination and degradation, or that SOCS1 binds to the Cul5–Rbx2 module too weakly to have been previously detected (Kamura et al., 2004). Recently, it was reported that SOCS1 and SOCS3 bind more weakly to Cul5, with affinities of 100- and 10-fold lower, respectively, than to Cul2 (Babon et al., 2009). In general, micromolar affinities are common in physiological interactions, and SOCS1 and SOCS3 have 1 and 0.1 μM affinities, respectively, for Cul5 (Babon et al., 2009). Therefore it is possible that all CIS/SOCS family members can act as ubiquitin ligases. This may explain why only SOCS1 and SOCS3 have been shown to suppress signaling using both SOCS box-dependent and -independent mechanisms (Babon et al., 2009).
FIGURE 5 | Domain organization of SOCS box proteins. (A) The SOCS box consists of a BC box and a Cul5 box in the order indicated. SH2, Src homology 2 phosphotyrosine binding domain; WD40, WD40 repeats; SPRY, sp1A/ryanodine receptor domain; Ank, ankyrin repeats; LRR, leucine-rich repeats; GTPase, GTPase domain. (B) Alignment of amino acid sequences of selected Cul5 boxes. Identical amino acids are highlighted in yellow. GenBank™ accession numbers of each protein are indicated. The consensus sequence is indicated below. Φ, hydrophobic residue.
production of NO (Lowenstein and Padalko, 2004). As a result, reactive nitrogen intermediates (such as NO, nitrite, and nitrate) and the products of the interaction of NO with reactive oxygen species (such as peroxynitrite and peroxynitrous acid) are accumulated and used to inhibit viruses or bacteria (Fang, 1997; Nathan and Shiloh, 2000; Lowenstein and Padalko, 2004). SSB1, 2 and 4 polyubiquitinate iNOS for proteasomal degradation (Kuang et al., 2010; Nishiya et al., 2011). SSB2-deficient macrophages showed prolonged iNOS and NO production, resulting in the enhanced killing of L. major parasites (Kuang et al., 2010). Further study showed that SSB1 and SSB4 are major ubiquitin ligases for iNOS and prevent the overproduction of NO, which could cause cytotoxicity (Nishiya et al., 2011).

**CRL5WSB1 COMPLEX**

WSB1 polyubiquitinates homeodomain-interacting protein kinase 2 (HIPK2), which is a nuclear protein kinase and is well-conserved from *Drosophila* to humans (Choi et al., 2005; 2008). HIPK2 interacts with a variety of transcription factors (D’Orazio et al., 2002; Hofmann et al., 2002; Zhang et al., 2005; Kim et al., 2006), the p300/CBP co-activator (Kim et al., 2002; Aikawa et al., 2006), and the Groucho/TLE co-repressor (Choi et al., 2005). The loss of HIPK2 reduces apoptosis and increases the numbers of trigeminal ganglia, while the overexpression of HIPK2 in the developing sensory and sympathetic neurons promotes apoptosis in a caspase-dependent manner (Doxakis et al., 2004; Wiggins et al., 2004). HIPK2 plays an important role in apoptosis mediated by p53, CtbP, Axin, Br3, Sp100, TP53INP1, and PML (Moller et al., 2003a,b; Tomasini et al., 2003; Doxakis et al., 2004; Kaneishii et al., 2004). UV irradiation activates and stabilizes HIPK2, most likely by WSB1-independent auto-phosphorylation, which results in the phosphorylation of p53 at Ser46. Expression of p53 target genes then promotes apoptosis (D’Orazio et al., 2002; Hofmann et al., 2002). Genotoxic stresses, such as adriamycin and cisplatin, also inhibit polyubiquitination of HIPK2 by WSB1 (Choi et al., 2008). HIPK2 also phosphorylates CtbP at Ser422 and phosphorylated CtbP is degraded via the 26S proteasome, resulting in apoptosis in p53-deficient cells (Zhang et al., 2003). WSB1 expression is induced by Sonic hedgehog (Shh) in developing limb buds and other embryonic structures (Vasilievskas et al., 1999). WSB1 also ubiquitinates the thyroid hormone-activating enzyme type II iodothyronine deiodinase (D2; Dentice et al., 2005). Ubiquitination of Shh-induced D2 by WSB1 induces parathyroid hormone-related peptide (PTHrP), thereby regulating chondrocyte differentiation (Dentice et al., 2005). In addition to HIPK2 and D2, WSB1 also binds to the interleukin-21 receptor (IL-21R; Nara et al., 2011). However, instead of promoting its degradation, WSB1 inhibits the degradation of the mature form of IL-21R (Nara et al., 2011). WSB1 associates with the intracytoplasmic region of IL-21R and enhances the maturation of IL-21R from a N-linked glycosylated form to a fully glycosylated mature form (Nara et al., 2011). These data indicate that WSB1 has important roles in both the maturation and the degradation of IL-21R.

**CRL5ASB COMPLEX**

ASB2, 3, 4, 6, 9, and 11 can all bind to Cul5–Rbx2 and form ubiquitin ligase complexes. Retinoic acid induces ASB2 in acute promyelocytic leukemia cells (Guibal et al., 2002). ASB2 targets the actin-binding proteins filamin A and B for proteasomal degradation (Heuze et al., 2008). Since knockdown of endogenous ASB2 in leukemia cells delays retinoic acid-induced differentiation and filamin degradation, ASB2 may regulate hematopoietic cell differentiation by targeting failamins for degradation and thereby modulating actin remodeling (Heuze et al., 2008). ASB2 and Skp2 associate with each other to bridge the formation of a non-canonical cullin1- and cullin5-containing dimeric ubiquitin ligase complex and promote the polyubiquitination and degradation of Jak3 (Nie et al., 2011; Wu and Sun, 2011).

Tumor necrosis factor receptor type 2 (TNF-R2) is polyubiquitinated by ASB3 for proteasomal degradation (Chung et al., 2005). ASB3 can affect T cell signaling by degrading TNF-R2, resulting in the inhibition of downstream signaling events in response to TNF-α (Chung et al., 2005).

Insulin receptor substrate 4 (IRS4) is an adaptor molecule involved in signal transduction by both insulin and leptin, and is widely expressed throughout the hypothalamus, with the greatest expression observed in the medial preoptic nucleus, ventromedial hypothalamus, and arcuate nucleus (Numan and Russell, 1999). ASB4 co-localizes and interacts with IRS4 in hypothalamic neurons (Li et al., 2011). ASB4 polyubiquitinates IRS4 for degradation and decreases insulin signaling (Li et al., 2011).

ASB6 is expressed in 3T3-L1 adipocytes but not in fibroblasts (Wilcox et al., 2004). ASB6 may regulate components of the insulin signaling pathway in adipocytes by promoting the degradation of adapter protein with a pleckstrin homology and SH2 domain (APS; Wilcox et al., 2004).

ASB9 polyubiquitinates creatine kinase B (CKB) and decreases total CKB levels (Debrincat et al., 2007).

The notch signaling pathway is essential for the spatio-temporal regulation of cell fate (Mumm and Kopan, 2000; Lai, 2004; Louvi and Artavanis-Tsokas, 2006). The single-pass transmembrane protein delta acts as a ligand for the notch receptor. *Danio rerio* Asb11 (d-Asb11) regulates compartment size in the endodermal and neuronal lineages via the ubiquitination and degradation of deltaA, leading to the activation of the canonical notch pathway (Diks et al., 2006, 2008). This recognition is specific to deltaA because d-Asb11 does not degrade deltaD (Diks et al., 2008). In zebrafish embryos, knockdown of d-Asb11 repressed specific delta–notch elements and their transcriptional targets, whereas these were induced when d-Asb11 was misexpressed (Diks et al., 2008). These data indicate that d-Asb11 regulates delta–notch signaling for the fine-tuning of lateral inhibition gradients between deltaA and notch (Diks et al., 2008).

**CRL5ASB40C COMPLEX AND CRL5MUF1 COMPLEX**

The substrates of Rab–40C and MUF1 have not yet been identified. However, Rab–40C localizes in the perinuclear recycling compartment, suggesting its physiological role in receptor endocytosis (Rodriguez-Gabin et al., 2004). Given that the mRNA and protein level of Rab-40C increases as oligodendrocytes differentiate, it may be important in myelin formation (Rodriguez-Gabin et al., 2004).
**VIRAL ECS-TYPE UBQUITIN LIGASE**

**CRL2<sup>HVIP/HEI</sup> COMPLEX**

Human papillomavirus (HPV) type 16 cause premalignant squamous intraepithelial neoplasia (Munger et al., 2004). Integration of viral DNA into the host genome leads to persistent and dysregulated expression of HPV E6 and E7 oncoproteins, which is necessary for the induction and maintenance of the oncogenic transformation (Munger et al., 2004). HPV E7 contains incomplete Cul2 box and can bind to endogenous Cul2 (Huh et al., 2007). HPV E7 polyubiquitinates retinoblastoma tumor suppressor (pRb) and induces proteasomal degradation (Boyer et al., 1996; Berezutskaya et al., 1997; Jones and Munger, 1997; Huh et al., 2007).

**CRL5<sup>VI</sup> COMPLEX**

The viral infectivity factor (Vif) protein of human immunodeficiency virus-1 (HIV-1; Yu et al., 2003) is also a Cul5-type ubiquitin ligase (Yu et al., 2003; Bergeron et al., 2010). Importantly, it has been suggested that the zinc-binding motif of Vif is important for its interaction with Cul5 (Yu et al., 2004; Mehle et al., 2006; Xiao et al., 2006). Vif polyubiquitinates and degrades the cellular intrinsic restriction factors APOBEC3F and APOBEC3G (Yu et al., 2003; Mehle et al., 2004; Liu et al., 2005). Both APOBEC3F and G have cytidine deaminase activity and, when packaged into HIV-1 virions, cause uracil (U) to be substituted for cytosine (C) in newly synthesized minus-strand viral DNA (Sheehy et al., 2002; Harris et al., 2003; Lecossier et al., 2003). These mutations cause amino acid substitutions, which affect the enzymatic activity of HIV-1 (Harris et al., 2003). Another possibility is that deoxyuridine in minus-strand viral DNA is targeted for enzymatic activity of HIV-1 (Harris et al., 2003). Another possibility is that deoxyuridine in minus-strand viral DNA is targeted for excision by uracil-DNA glycosylase (Harris et al., 2003). These basic sites are recognized and cleaved by endonucleases, inhibiting HIV-1 replication (Harris et al., 2003). Since the CRL5<sup>VI</sup> complex targets APOBEC3F and APOBEC3G for proteasomal degradation, it is a potential target for the development of antiviral agents aimed at preventing the interaction between Vif and Cul5.

**CRL5<sup>E4orf6</sup> COMPLEX**

The human adenovirus type 5 (Ad5) early region 4 34-kDa product from open reading frame 6 (E4orf6) contains three BC boxes (Blanchette et al., 2004; Cheng et al., 2007, 2011). Although Ad5 E4orf6 forms complex containing Cul5, Elongin BC complex, and Rbx1, Cul5 box is not present in the Ad5 E4orf6 (Harada et al., 2002; Blanchette et al., 2004; Cheng et al., 2011). Adenoviral protein E1B55K associates with the E4orf6 protein and recognizes substrate to be degraded by ubiquitin–proteasome pathway (Blanchette et al., 2004; Cheng et al., 2007; Luo et al., 2007). This complex is essential for efficient viral replication and some substrates have been identified, including p53 (Moore et al., 1996; Querido et al., 1997; Steegenga et al., 1998; Cathomen and Weitzman, 2000; Nevels et al., 2000; Shen et al., 2001), meiotic recombination 11 (Mre11; Stracker et al., 2002; Blanchette et al., 2004), DNA ligase IV (Baker et al., 2007), integrin α3 (Dallaire et al., 2009), and adeno-associated virus type 5 (AAV5) Rep52 and capsid proteins (Nayak et al., 2008). The Mre11 complex consists of Mre11, RAD50, and Nijmegen breakage syndrome 1 (NBS1, also known as nibrin) is a sensor of DNA double-strand breaks (DSBs) and induces p53-dependent apoptosis (Stracker and Petrini, 2011). DNA ligase IV plays a pivotal role in repairing DSBs and the mutation of this gene results in ligase IV (LIG4) syndrome characterized by pronounced radiosensitivity, genome instability, malignancy, immunodeficiency, and bone marrow abnormalities (Chistjakov et al., 2009). Heterodimer of integrin α and β subunits functions as transmembrane receptor that links external ligands to intracellular signaling pathways. Integrin αβ3 heterodimer in which the α3 subunit is coupled to the β1 subunit binds a variety of extracellular matrix substrates, including fibronectin, collagen, vitronectin, and laminins (DiPersio et al., 1995). E4orf6/E1B55K ligase complex is

**Table 1 | Cul2-type ubiquitin ligases and corresponding substrates.**

| Ubiquitin ligase | Substrates | References |
|-----------------|------------|------------|
| pVHL | HiFa | Ivan et al. (2001); Jaaikola et al. (2001); Masson et al. (2001); Hon et al. (2002) |
| Spry2 | EGFFR | Zhou and Yang (2011) |
| Atypical PKC (PKCζ) and (η) | Okuda et al. (2001); Iturrioz and Parker (2007) |
| RPB7 | Na et al. (2003) |
| Rpb1 | Kuznetsova et al. (2003); Mikhaylova et al. (2003) |
| LRR-1 | CKI-1 (in C. elegans) | Starostina et al. (2010) |
| FEM1B | p21<sup>Cip1</sup> | Starostina et al. (2010) |
| FEM1B | TRA-1 | Starostina et al. (2007) |
| FEM1B | Ankrd37 | Shi et al. (2011) |

**Table 2 | Cul5-type ubiquitin ligases and corresponding substrates.**

| Cul5-type ubiquitin ligases | Substrates | References |
|-----------------------------|------------|------------|
| SOCS1 | JAK2 | Kamizono et al. (2001) |
| ElonginA | Rbp1 | Yasukawa et al. (2008) |
| SSB1, 2, and 4 | iNOS | Kuang et al. (2010); Nishiyama et al. (2011) |
| WSB1 | HIPK2 | Choi et al. (2005, 2008) |
| ASB2 | Filamin A and B | Heuze et al. (2008) |
| ASB3 | TNF-R2 | Chung et al. (2005) |
| ASB4 | IRS4 | Li et al. (2011) |
| ASB6 | APS | Wilcox et al. (2004) |
| ASB9 | CKB | Debrincat et al. (2007) |
| ASB11 | DeltaA (in Danio rerio) | Diks et al. (2006, 2008) |
Table 3 | Viral ECS-type ubiquitin ligases and corresponding substrates.

| Viral ECS-type ubiquitin ligases | Substrates | References |
|----------------------------------|------------|------------|
| HPV16E7 (Cul2-type)              | pRB        | Boyer et al. (1996); Berezutskaya et al. (1997); Jones and Munger (1997); Huh et al. (2007) |
| Vif (Cul5-type)                  | APOBEC3F and APOBEC3G | Yu et al. (2003); Mehle et al. (2004); Liu et al. (2005) |
| E4orf6 of Ad5 (Cul5-type)        | p53        | Moore et al. (1996); Querido et al. (1997); Steegenga et al. (1998); Cathomen and Weitzman (2000); Nevels et al. (2000); Shen et al. (2001) |
| Mre11                            | DNA ligase IV | Stracker et al. (2002); Blanchette et al. (2004) |
| Integrin α3                      |            | Baker et al. (2007); Dallaire et al. (2009) |
| AAV5 Rep52 and capsid proteins   |            | Nayak et al. (2008); Cheng et al. (2011) |
| DNA ligase IV                    | p53        | Sato et al. (2009a,b) |

involved in cell detachment from the extracellular matrix, which may contribute to virus spread (Dallaire et al., 2009). Although Cul5 is present in the E4orf6 complex of the human Ad5, Cul2 is primarily present in the E4orf6 complex of Ad12 and Ad40 (Cheng et al., 2011). Interestingly, E4orf6 complex of Ad16 binds Cul2 as well as Cul5 and is not able to degrade p53 and integrin α3 (Cheng et al., 2011). It remains unclear how E4orf6 complexes of each serotype distinguish Cul2 from Cul5.

CRL5(ZEBRA) COMPLEX

Epstein–Barr virus (EBV), a human γ-herpesvirus, is associated with several B cell and epithelial cell malignancies and there are two different infection states, latent, and lytic (Tsurumi, 2001). BZLF1 (known as Zta, EB1, or ZEBRA), is a transcriptional activator that induces EBV early gene expression to activate an EBV lytic cycle cascade (Chevallier-Greco et al., 1986; Countryman et al., 1987; Hammerschmidt and Sugden, 1988; Sinclair et al., 1991). BZLF1 can bind to Cul2 and Cul5 because of presence of both Cul2 and Cul5 boxes (Sato et al., 2009a). BZLF1 polyubiquitinitates and induces degradation of p53 (Sato et al., 2009a,b), The degradation of p53 prevents apoptosis and is required for the efficient viral propagation in the lytic replication.

CONCLUSION

The “classical” SOCS box proteins can be divided into two distinct families. Cul2 and Cul5 within the VHL box and SOCS box, respectively, determine the association with Rbx1 or Rbx2. Given that Rbx1 and Rbx2 specifically interact with Cul2 and Cul5, respectively, the functions of Rbx1 and Rbx2 are different from each other, at least in higher eukaryotes. Cul2- and Cul5-type ubiquitin ligases are structurally similar because they have the Elongin BC complex adaptor protein and Cullin scaffold protein in common. As with other ubiquitin ligases, these two have various substrates and physiological functions (Tables 1, 2, and 3) and may have arisen independently during evolution.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid from Scientific Research on Innovative Areas and grants from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

Aikawa, Y., Nguyen, L. A., Isono, K., Takakura, N., Tagata, Y., Schmitz, M. L., Koseki, H., and Kitabayashi, I. (2006). Roles of HBP1 and HBP2 in AML1- and p300-dependent transcription, hematopoiesis and blood vessel formation. EMBO J. 25, 3955–3965.

Anderson, K., Nordquist, K. A., Gao, X., Hicks, K. C., Zhai, B., Gygi, S. P., and Patel, T. B. (2011). Regulation of cellular levels of Sprouty2 by prolyl hydroxylase domain proteins and von-Hippel Lindau protein. J. Biol. Chem. 286, 42027–42036.

Aravind, L., and Koonin, E. V. (2000). The U box is a modified RING finger – a common domain in ubiquitination. Curr. Biol. 10, R132–R134.

As with other ubiquitin ligases, these two have various substrates and physiological functions (Tables 1, 2, and 3) and may have arisen independently during evolution.

As with other ubiquitin ligases, these two have various substrates and physiological functions (Tables 1, 2, and 3) and may have arisen independently during evolution.

Baker, A., Rohleder, K. J., Hanakahi, L. A., and Ketner, G. (2007). Adenovirus E4 34k and E1b 55k oncoproteins target host DNA ligase IV for proteasomal degradation. J. Virol. 81, 7034–7040.

Berezutskaya, E., Yu, B., Morozov, A., Raychaudhuri, P., and Bagchi, S. (1997). Differential regulation of the pocket domains of the retinoblastoma family proteins by the HPV16 E7 oncoprotein. Cell Growth Differ. 8, 1277–1286.

Bergeron, J. R., Huthoff, H., Velkov, D. A., Beavil, R. L., Simpson, P. J., Matthews, S. J., Malim, M. H., and Sanderson, M. R. (2010). The SOCS-box of HIV-1 Vif interacts with Elongin BC by induced-folding to recruit its Cul5-containing ubiquitin ligase complex. PLoS Pathog. 6, e1000925. doi:10.1371/journal.ppat.1000925

Bhattacharya, A., Deng, J. M., Zhang, Z., Behringer, R., de Crombrugghe, B., and Maity, S. N. (2003). The B subunit of the CCAAT-box binding transcription factor complex (CBF/NF-Y) is essential for early mouse developmental and cell proliferation. Cancer Res. 63, 8167–8172.

Blanchette, P., Cheng, C. Y., Yan, Q., Ketner, G., Ornellses, D. A., Dobner, T., Conaway, R. C., Conaway, J. W., and Branton, P. E. (2004). Both BC-box motifs of adenovirus protein E4orf6 are required to efficiently assemble an E3 ligase complex that degrades p53. Mol. Cell. Biol. 24, 9619–9629.

Boyer, S. N., Wazer, D. E., and Band, V. (1996). E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. Cancer Res. 56, 4620–4624.
mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc. Natl. Acad. Sci. U.S.A. 96, 11364–11369.
Louve, A., and Artavanis-Tsakonas, S. (2006). Notch signalling in vertebrate neural development. Nat. Rev. Neurosci. 7, 93–102.
Lowenstein, C. J., and Padalia, E. (2004). iNOS (NOS2) at a glance. J. Cell Sci. 117, 2865–2867.
Luo, K., Ehrlich, E., Xiao, Z., Zhang, W., Ketner, G., and Yu, X. F. (2007). Okumura et al. Elongin BC–Cul2/5-containing ubiquitin ligases Adenovirus E4orf6 assembles with Cullin5-ElonginB-ElonginC E3 ubiquitin ligase through an HIV/SIV Vif-like BC-box to regulate p53. FAseB J. 21, 1742–1750.
Mahrour, N., Redwine, W. B., Florens, L., Swanson, S. K., Martin-Brown, S., Bussiere-Wu, L. D., Stachura, Hampton, K., Washburn, M. P., Conaway, R. C., and Conaway, J. W. (2008). Characterization of cullin-box sequences that direct recruitment of Cul-2-Rbx1 and Cul-5-Rbx2 modules to Elongin BC-based ubiquitin ligases. J. Biol. Chem. 283, 8005–8013.
Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., and Trono, D. (2003). Broad antietriviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 424, 99–103.
Mariani, R., Chen, D., Schrofelbauer, B., Farova, K., Ronig, B., Bollman, B., Munk, C., Nymark-McMahon, H., and Landau, N. R. (2003). Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 114, 21–31.
Masson, N., Willam, C., Maxwell, P. H., Pugh, C. W., and Ratcliffe, P. J. (2001). Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J. 20, 5197–5206.
Maxwell, P. H., Wiesener, M. S., Chang, G. W., Clifford, S. C., Vaux, E. C., Cockman, M. E., Wykoff, C. C., Pugh, C. W., Maher, E. R., and Ratcliffe, P. J. (1999). The tumour suppressor protein VHL contains a hypoxia-inducible factor-alpha box that triggers oxygen-dependent proteolysis. Nature 399, 271–275.
Mehle, A., Goncalves, J., Santa-Marta, M., McPike, M., and Gabuzda, D. (2004). Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev. 18, 2861–2866.
Mehle, A., Thomas, E. R., Rajendran, K. S., and Gabuzda, D. (2006). A zinc-binding region in Vif binds Cul5 and determines culin selection. J. Biol. Chem. 281, 17259–17265.
Mikhalysova, O., Ignacik, M. L., Baranikiewicz, T. J., Harbaugh, S. V., Yi, Y., Maxwell, P. H., Schneider, M., Van Geyte, K., Carmeleit, P., Revelo, M. P., Wyder, M., Greis, K. D., Meller, J., and Czyzyk-Krzeska, M. F. (2008). The von Hippel-Lindau tumor suppressor protein and Egl-9-Type proline hydroxylases regulate the large subunit of RNA polymerase II in response to oxidative stress. Mol. Cell. Biol. 28, 2701–2717.
Moller, A., Sirma, H., Hofmann, T. G., Rueler, S., Klimczak, E., Droge, W., Will, H., and Schmitz, M. L. (2003a). PML is required for human papillomavirus-interacting protein kinase 2 (HPK2)-mediated p53 phosphorylation and cell cycle arrest but is dispensable for the formation of HPK2 domains. Cancer Res. 63, 4310–4314.
Moller, A., Sirma, H., Hofmann, T. G., Staege, H., Greko, E., Ludi, K. S., Klimczak, E., Droge, W., Will, H., and Schmitz, M. L. (2003b). Sp100 is important for the stimulatory effect of homeodomain-interacting protein kinase-2 on p53-dependent gene expression. Oncogene 22, 8731–8737.
Moore, M., Horikoshi, N., and Shenk, T. (1996). Oncogenic potential of the adenosine Efor6 protein. Proc. Natl. Acad. Sci. U.S.A. 93, 11295–11301.
Mumm, J. S., and Kopan, R. (2000). Notch signaling: from the outside in. Dev. Biol. 228, 151–165.
Munger, K., Baldwin, A., Edwards, K. M., Hayakawa, M., Nguyen, C. L., Owens, M., Grace, M., and Huh, K. (2004). Mechanisms of human papillomavirus-induced oncogenesis. J. Virol. 78, 11451–11460.
Na, X., Duan, H. O., Messing, E. M., Schoen, S. R., Ryan, C. K., di Sant’Agnese, P. A., Golemis, E. A., and Wu, G. (2003). Identification of the RNA polymerase II subunit inRPB7 as a novel target of the von Hippel-Lindau protein. EMBO J. 22, 4249–4259.
Naka, T., Narakazi, H., Hirata, M., Matsumoto, T., Minamoto, S., Aono, A., Nishimoto, N., Kajita, T., Taga, T., Yoshikazi, H., Akira, S., and Kishimoto, T. (1997). Structure and function of a new STAT-binding domain associated with activated STAT protein kinase c. J. Biol. Chem. 272, 43611–43617.
Parkinson, S. J., Le Good, J. A., Whelan, R. D., Whitehead, P., and Parker, P. J. (2004). Identification of PKCzetall: an endogenous inhibitor of cell polarity. EMBO J. 23, 77–88.
Pauw, A., Lee, S., Worrell, R. A., Chen, D. Y., Burgess, W. H., Linehan, W. M., and Klausner, R. D. (1997). The von Hippel-Lindau tumor suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. Proc. Natl. Acad. Sci. U.S.A. 94, 2156–2161.
Peters, J. M. (1998). SCF and Apc: the Yin and Yang of cell cycle regulated proteolysis. Curr. Opin. Cell Biol. 10, 759–768.
Pissevaux, J., Lavens, D., Peelman, F., and Tavernier, J. (2008). The many faces of the SOCS box. Cytokine Growth Factor Rev. 19, 371–381.
Querido, E., Marcellus, R. C., Lai, A., Charbonneau, K., Teodoro, I. G., Ketner, G., and Branton, P. E. (1997). Regulation of p53 levels by the E1B 55-kilodalton protein and E4orf6 in adeno-virus-infected cells. J. Virol. 71, 3788–3798.
Ram, P. A., and Waxman, D. J. (1999). SOCS/CIS protein inhibition of growth hormone-stimulated STAT5 signaling by multiple mechanisms. J. Biol. Chem. 274, 35553–35561.
Rodriguez-Gabin, A. G., Almazan, G., and Larocca, J. N. (2004). Vesicle transport in oligodendrocytes: probable role of Rab40c protein. J. Neurosci. Res. 78, 758–770.
Rui, L., Yuan, M., Frantz, D., Shoelson, S., and White, M. F. (2002). SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J. Biol. Chem. 277, 42394–42398.
Sartori da Silva, M. A., Tee, J. M., Pari-daen, J., Brouwers, A., Runtuwe, Z., Zivkovic, D., Diks, S. H., Guar-davaccaro, D., and Peppelenbosch, M. P. (2010). Essential role for the d-Asb11 cul5 Box domain for proper notch signaling and neural cell fate decisions in vivo. PLoS ONE 5, e14023. doi:10.1371/journal.pone.0014023
Sato, Y., Kamura, T., Shirata, N., Murata, T., Kudoh, A., Ishwari, S., Nakayama, S., Isomura, H., Nishiyama, Y., and Turumi, T. (2009a). Degradation of phosphorylated p53 by viral protein-ECS E3 ligase complex. PLoS Pathog. 5, e1000530. doi:10.1371/journal.ppat.1000530
Sato, Y., Shirata, N., Kudoh, A., Ishwari, S., Nakayama, S., Murata, T., Isomura, H., Nishiyama, Y., and Turumi, T. (2009b). Expression of Epstein-Barr virus BZLF1 immediate-early protein induces p53 degradation independent of MDM2, leading to repression of p53-mediated transcription. Virology 388, 204–211.
Schefﬂer, M., Nuber, U., and Hubertgurt, J. M. (1995). Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature 373, 81–83.
Okumura et al. Elongin BC–Cul2/5-containing ubiquitin ligases

Introduction

Elongin BC (Elp1 and Elp2) is a member of the Elongin family, which plays a critical role in regulating the stability of various proteins. The Elongin BC–Cul2/5-containing ubiquitin ligase complex (ECCL) is involved in the degradation of several important proteins, including p53, which plays a crucial role in the regulation of cell cycle progression and apoptosis. The ECCL has been shown to interact with the E3 ubiquitin ligase with a dockerin (Ubl-DIN) domain (Urbano et al., 2010). The ECCL has also been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53. The ECCL has also been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53.

Materials and Methods

Cell culture and transfection

HeLa cells were grown in DMEM containing 10% FBS and 1% Penicillin-Streptomycin (Sigma). Transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

Results

The ECCL complex has been shown to be involved in the degradation of several important proteins, including p53. The ECCL has been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53. The ECCL has also been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53.

Discussion

The ECCL complex has been shown to be involved in the degradation of several important proteins, including p53. The ECCL has been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53. The ECCL has also been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53.

Conclusion

In conclusion, the ECCL complex has been shown to be involved in the degradation of several important proteins, including p53. The ECCL has been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53. The ECCL has also been shown to interact with the Cul5 protein, which is involved in the degradation of several proteins, including p53.

Conflict of Interest Statement:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 November 2011; paper pending published: 12 December 2011; accepted: 17 January 2012; published online: 03 February 2012.

Citation: Okumura F, Matsuzaki M, Nakatsuka K and Kamura T (2012) The role of Elongin BC-containing ubiquitin ligase complexes in the degradation of the activated EGFR. Prog. ONE 6, e2936. doi: 10.1371/journal.pone.0029396

This article was submitted to Frontiers in Molecular and Cellular Oncology, a specialty of Frontiers in Oncology.

Copyright © 2012 Okumura, Matsuzaki, Nakatsuka and Kamura. This is an open-access article distributed under the terms of the Creative Commons Attribution Non Commercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.