HECT and RING finger families of E3 ubiquitin ligases at a glance

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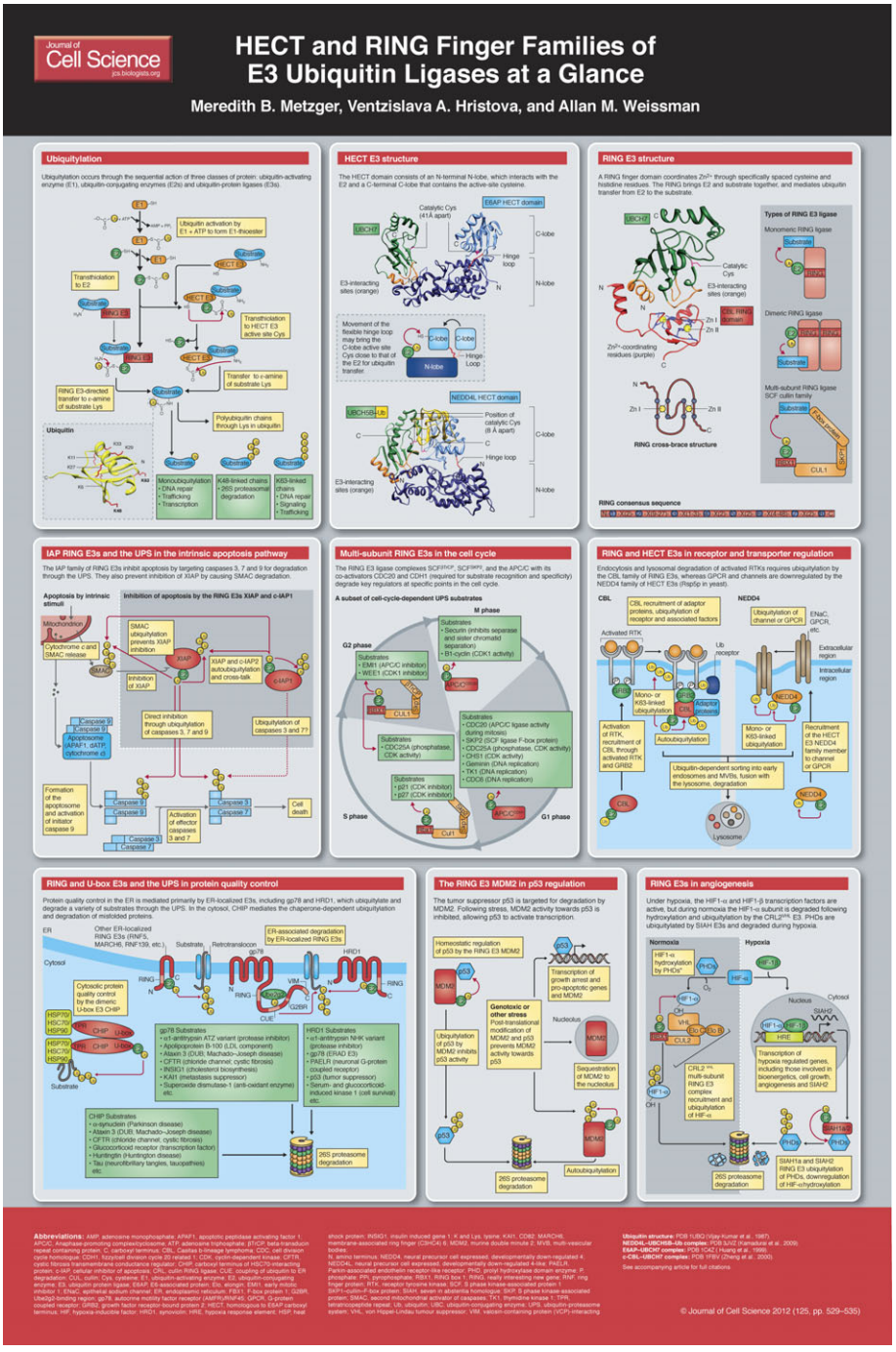
This article is part of a Minifocus on Ubiquitin. For further reading, please see related articles: 'Ubiquitin and SUMO in DNA repair at a glance' by Helle D. Ulrich (*J. Cell Sci.* **125**, 249-254), 'Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control' by Annamaria Mocciaro and Michael Rape (*J. Cell Sci.* **125**, 255-263), 'The role of ubiquitylation in receptor endocytosis and endosomal sorting' by Kaisa Haglund and Ivan Dikic (*J. Cell Sci.* **125**, 265-275), 'Cellular functions of the DUBs' by Michael J. Clague et al. (*J. Cell Sci.* **125**, 277-286), 'Non-canonical ubiquitin-based signals for proteasomal degradation' by Yelena Kravtsova-Ivantsiv and Aaron Ciechanover (*J. Cell Sci.* **125**, 539-548) and 'No one can whistle a symphony alone – how different ubiquitin linkages cooperate to orchestrate NF-κB activity' by Anna C. Schmukle and Henning Walczak (*J. Cell Sci.* **125**, 549-559).

The post-translational attachment of ubiquitin, a highly conserved 76-amino-acid polypeptide, directs myriad eukaryotic proteins to a variety of fates and functions. Ubiquitylation is best-known for targeting proteins for degradation by the 26S proteasome. Other functions include internalization and lysosomal targeting, modulation of protein interactions, alteration of subcellular distribution, regulation of transcription, DNA repair and propagation of transmembrane signaling, most notably in the NF-κB pathway. Not surprisingly, ubiquitylation has been linked to virtually every cellular process.

Ubiquitylation occurs through the sequential action of three classes of protein: ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s, also referred to as ubiquitin carrier proteins or UBCs), and ubiquitin-protein ligases (E3s) (Komander, 2009). The C-terminal carboxyl group of ubiquitin is activated in an ATP-dependent process, which results in a high-energy thioester linkage with the active-site cysteine of E1. Ubiquitin is trans-thiolated from the E1 to the active-site cysteine of one of ~40 E2s (in mammals). Finally, ubiquitin is generally transferred from the E2 to the ε-amino group of a substrate lysine in an E3-dependent manner. For the E3 ligases of the homologous to the E6AP carboxyl terminus (HECT) domain family (Rotin and Kumar, 2009), this involves an obligate thioester intermediate with the active-site cysteine of the E3. The vast majority of E3 ligases belong to the group of really interesting new gene (RING) and RING-related E3s – such as plant homeodomain (PHD) and leukemia associated protein (LAP) finger proteins, and members of the U-box family (Deshaies and Joazeiro, 2009). These mediate the direct transfer of ubiquitin from E2 to substrate. Ubiquitin can also be added to the N-terminus of proteins (Ciechanover and Ben-Saadon, 2004), and the modification of other nucleophilic amino acids (threonine, serine, cysteine) by ubiquitylation has been described (Cadwell and Coscoy, 2005; Carvalho et al., 2007; Ishikura et al., 2010; Shimizu et al., 2010; Tait et al., 2007; Wang et al., 2007; Williams et al., 2007).

Substrates can be modified by a single ubiquitin on one or more sites (monoubiquitylation and multi-monoubiquitylation, respectively) or can be tagged with chains of ubiquitin (poly- or multiubiquitylation). The



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majority of experimental evidence suggests that chains are built sequentially, beginning with the substrate-attached ubiquitin, although there is evidence for chains being built on E2 or E3 and transferred to substrates en bloc (Bazirgan and Hampton, 2008; Li et al., 2007; Ravid and Hochstrasser, 2007; Wang and Pickart, 2005). Chain formation can occur through the seven internal lysine residues on ubiquitin or its N-terminus (Vijay-Kumar et al., 1987; Behrends and Harper, 2011; Clague and Urbe, 2010). The type of ubiquitin modification specifies the function of the modification (i.e. monoubiquitylation or different chain linkages), together with other factors such as ubiquitin receptors and the opposing action of deubiquitylating enzymes (DUBs); see also the Commentary by Michael J. Clague, Judy M., Coulson and Sylvie Urbé in the previous issue (*J. Cell Sci.* **125**, xxxx).

Ubiquitylation occurs with exquisite spatial, temporal and substrate specificity, much of which is dictated by the more than 600 E3s that are estimated to be encoded by the mammalian genome (Li et al., 2008). In addition to this cellular specificity and complexity, E3s are implicated in a number of pathophysiological conditions, which makes them attractive therapeutic targets (Kirkin and Dikic, 2011; Lipkowitz and Weissman, 2011). This Cell Science at a Glance article and the accompanying poster describe structural aspects of E3s, and highlight several mammalian pathways and cellular processes in which E3s have key roles. The roles of other specific E3s and cellular processes that are regulated by ubiquitylation are discussed in detail in accompanying review articles in this and the previous issue.

HECT domain E3s

In mammals, there are ~30 HECT domain E3s. Among their many functions, HECT E3s have prominent roles in protein trafficking, the immune response, and in several signaling pathways that regulate cellular growth and proliferation (Rotin and Kumar, 2009). The conserved HECT domain (which comprises ~350 amino acids) is located at the C-terminus of these enzymes, whereas their N-terminal domains are diverse and mediate substrate targeting. The HECT domain itself is bi-lobed, consisting of an N-terminal N-lobe that interacts with the E2 and a C-terminal C-lobe that contains the active-site cysteine that forms the thioester with ubiquitin (Huang et al., 1999). The crystal structure of the NEDD4L HECT domain in complex with ubiquitin-conjugated E2 [UBCH5B~Ub, officially known as UBE2D2] shows the C-lobe contacting the esterified ubiquitin and folding down onto UbcH5B, thereby making the distance between the E2 and E3 catalytic cysteines ~8Å (Kamadurai et al., 2009). By contrast, the first identified member of the HECT family, E6-associated protein (E6AP, officially known as UBE3A) (Huibregtse et al., 1995), in complex with its E2 UBCH7 (officially known as UBE2L3), shows the C-lobe in a more open architecture where the catalytic cysteine residues are 41Å apart (Huang et al., 1999). Taken together, these and other structural studies (Ogunjimi et al., 2005; Verdecia et al., 2003) suggest that the two lobes of the HECT domain are connected through a flexible hinge that allows them to come together during ubiquitin transfer. Interestingly, ubiquitin chain linkage specificity appears to be inherently dependent on the last 60 amino acids of the HECT domain C-lobe (Kim and Huibregtse, 2009).

RING finger E3s

The mammalian genome encodes more than 600 potential RING finger E3s (Li et al., 2008). A canonical RING finger is a Zn^{2+}-coordinating domain that consists of a series of specifically spaced cysteine and histidine residues, and facilitates E2-dependent ubiquitylation (Lorick et al., 1999). The structure of the RING domain of Cbl in complex with an E2 illustrates several notable features of the RING domain (Zheng et al., 2000). The two zinc ions and the coordinating residues form a 'cross-brace' structure. Unlike the HECT domain, the RING finger domain does not form a catalytic intermediate with ubiquitin. Instead, the RING finger serves, at a minimum, as a scaffold that brings E2 and substrate together, and at least one study suggests that RING finger domains can also allosterically activate E2s (Ozkan et al., 2005).

Members of the RING finger ubiquitin ligase family can function as monomers, dimers or multi-subunit complexes. Dimerization generally occurs through the RING finger domain or surrounding regions and can result in homodimers [e.g. cIAP (cellular inhibitor of apoptosis; officially known as BIRC2), RNF4 (ring finger protein 4), SIAH (seven in absentia homologue 1), and TRAF2 (TNF receptor-associated factor 2)] (Liew et al., 2010; Mace et al., 2008; Park et al., 1999; Polekhina et al., 2002) or heterodimers [e.g. MDM2 (murine double minute 2, also known as HDM2 in human) and MDMX (officially known as MDM4, also known as HDMX or HDM4 in human), BRCA1 (breast cancer 1) and BARD1 (BRCA1-associated RING domain 1), RING1b (officially known RNF2) and BMI1 (BMI1 polycomb ring finger oncogene)] (Brzovic et al., 2001; Buchwald et al., 2006; Linke et al., 2008; Satijn and Otte, 1999; Sharp et al., 1999; Simons et al., 2006; Wu et al., 1996). For heterodimers, one RING domain (MDMX, BARD1, BMI1) often lacks ligase activity and might conform and/or stabilize the active E2-binding RING domain. Multi-subunit RING domains are exemplified by the cullin RING ligase (CRL) superfamily (Hua and Vierstra, 2011; Petroski and Deshaies, 2005), which includes the SCF complex, consisting of S-phase kinase-associated protein 1 (SKP1), cullin and F-box protein, and the more elaborate anaphase-promoting complex/cyclosome (APC/C).

Multi-subunit RING E3s and the cell cycle

Correct progression of the cell cycle is highly dependent on two families of multi-subunit RING E3s, the APC/C and the SCF; for a detailed discussion please also refer to the Commentary by Annamaria Mocchiaro and Michael Rape in the previous issue (*J. Cell Sci.* **125**, xxxx). The APC/C consists of at least thirteen different subunits, including the E2-binding RING finger protein APC11. One of two co-activators, cell division cycle homologue 20 (CDC20) and CDH1 (officially known as FZR1) confers substrate specificity by associating with the APC/C at specific stages of the cell cycle (Nakayama and Nakayama, 2006; Peters, 2006). APC/C^{CDC20} is active from prometaphase to telophase. Among its ubiquitin-proteasome system (UPS) substrates are securin, which enables correct separation of sister chromatids, and cyclin B, whose degradation is required to prevent ongoing activity of cyclin-dependent kinase 1 (CDK1) after the completion of mitosis (Peters, 2006). In G1 phase, CDH1 replaces CDC20 in APC/C and a second set of substrates is targeted for degradation. Interestingly, these include CDC20 and a substrate recognition element of the SCF family, the S-phase kinase-associated protein 2 (SKP2), which is also involved in cell cycle regulation (see below). The phosphatase CDC25 is also degraded by the UPS through the activity of APC/C^{CDH1}, which results in activation of the CDK inhibitors p21 and p27. Transition into S phase is facilitated by inactivation of CDH1 through phosphorylation by CDK2 (leading to its dissociation from the core APC/C) and targeting for degradation by both APC^{CDH1} and an unknown SCF E3 (Peters, 2006). APC/C activity is further regulated through association with its inhibitory pseudo-substrate early mitotic inhibitor (EMI1). EMI1 facilitates inactivation of APC/C from late G1 through S phase, and in some cells into G2 phase (Frescas and Pagano, 2008; Peters, 2006).

A second RING E3 complex that is required for cell cycle progression belongs to the SCF family of CRL E3s. SCF E3s utilize inter-

changeable F-box substrate-recognition elements and, therefore, there are potentially 69 different mammalian SCF E3s. Two F-box proteins implicated in cell cycle regulation are SKP2 and beta-transducin repeat-containing protein (β-TrCP, officially known as BTRC) (Frescas and Pagano, 2008). SCF^{SKP2} is active during S and G2 phases, and is responsible for the UPS-mediated degradation of p21 and p27, thereby leading to activation of CDK1 and CDK2. $SCF^{\beta\text{-}TrCP}$ targets CDC25A and WEE1 (another phosphatase) for degradation during G2, thereby exerting both a positive and a negative effect on CDK1 activity, respectively. $SCF^{\beta\text{-}TrCP}$ is also responsible for the degradation of EMI1 in late G2 phase (Frescas and Pagano, 2008).

The role of the RING E3 ligase MDM2 in the regulation of p53

The tumor suppressor protein p53 is a transcription factor that induces cell cycle arrest and apoptosis in response to stress such as DNA damage. Maintaining low basal levels of p53 without disrupting cell cycle progression requires a high degree of regulation, which is mediated by the UPS. Over ten E3s have been associated with the regulation of p53, but the RING E3 MDM2 is of unquestionable importance (Lee and Gu, 2010; Wade et al., 2010). MDM2 ubiquitylates p53 in a RING-finger-dependent manner, which leads to the proteasomal degradation of p53 (Fang et al., 2000; Haupt et al., 1997; Honda et al., 1997; Honda and Yasuda, 2000; Kubbutat et al., 1997). MDM2 also autoubiquitylates, thereby regulating its own level in the cell (Fang et al., 2000). The functional MDM2 E3 ligase can be a MDM2 homodimer or a heterodimer with MDMX (Lee and Gu, 2010; Wade et al., 2010). MDMX, itself, is essential for the regulation of p53 and binds to p53 in a manner that is similar to its binding to MDM2.

In response to genotoxic and other stresses, MDM2 activity towards p53 is inhibited through several mechanisms, which include phosphorylation of MDM2 and p53; these lead to increased p53 signaling. Furthermore, levels of the tumor suppressor ARF increase in response to stress, which results in MDM2 being sequestered into the nucleolus and its interaction with p53 being inhibited (Linke et al., 2008).

HECT and RING finger E3s, and their function in receptor and transporter regulation

Another process that is essential for the correct regulation of cell growth is the downregulation of receptor tyrosine kinase (RTK) signaling, which is accomplished in part by internalization of the ligand-activated RTK and its trafficking to the lysosome for degradation. Although the precise endocytic mechanism varies for different mammalian receptors, ubiquitylation by the CBL family of RING finger E3s has crucial roles in this process (Acconcia et al., 2009). In addition to the RING finger domain E3, CBL proteins contain a tyrosine-kinase-binding (TKB) domain that mediates direct binding to activated RTKs. CBL proteins can be recruited to RTKs indirectly through interactions with the adaptor protein growth-factor-receptor-bound protein 2 (GRB2) (Peschard et al., 2001). CBL family members modify the activated RTK with mono- or K63-linked ubiquitin chains that – together with the direct recruitment of proteins to CBL itself – assemble a multi-protein complex that includes ubiquitin-binding domain (UBD)-containing proteins (so-called ubiquitin receptors) (Swaminathan and Tsygankov, 2006). The exact role for RTK ubiquitylation by CBL in the initial receptor internalization step is unclear, but on-going ubiquitylation of RTKs by this E3 ligase is necessary for correct downstream sorting and degradation of the signaling complex by the lysosome (Marmor and Yarden, 2004; Williams and Urbe, 2007). As part of the process of RTK downregulation, CBL proteins are negatively regulated through autoubiquitylation. CBLs themselves are also subject to ubiquitylation by the HECT E3s, NEDD4 and ITCH (Magnifico et al., 2003), although the relationship between this ubiquitylation and RTK activation is unknown. Ubiquitylation, as well as de-ubiquitylation of components of the RTK complex and recruitment of ubiquitin-binding proteins, thus, have essential roles in the modulation of growth factor signaling.

The nine members of the NEDD4 family of HECT E3 ligases include the NEDD4 isoforms, ITCH and members of the SMAD-specific E3 ubiquitin protein ligases (SMURFs), which – collectively – are the mammalian orthologues of yeast Rsp5p. Early discoveries regarding endocytosis and vacuolar targeting in yeast advanced our understanding of the mechanisms that are involved in the downregulation of proteins from the plasma membrane in mammalian cells. The NEDD4 isoforms in particular mediate ubiquitylation that leads to the cell-surface downregulation of transporters and receptors, such as the epithelial sodium channel (ENaC) (Rotin and Kumar, 2009; Staub et al., 2000) and the β2-adrenergic receptor (ADRB2) (Shenoy et al., 2008). A common feature of the NEDD4 family is the presence of two to four double tryptophan residue (WW) domains in the N-terminal half of the molecule that recognize proline-containing (so-called PY) motifs on substrates (Rotin and Kumar, 2009). Most NEDD4 family members also contain an N-terminal C2 domain that facilitates interactions with the plasma membrane (Rotin and Kumar, 2009). Mutations in ENaC subunits that decrease the interaction with the WW domains of NEDDL are associated with Liddle syndrome, an autosomal dominant disorder that is characterized by severe hypertension (Rotin and Staub, 2011).

RING finger and U-box E3s, and the UPS in protein quality control

RING E3s mediate protein quality control by ubiquitylating an array of misfolded and unassembled proteins, as well as those whose levels must be regulated. In the endoplasmic reticulum (ER), the polytopic mammalian ER-associated degradation (ERAD) RING E3s gp78 (officially known as AMFR) and HRD1 (officially known as SYVN1) tag proteins with polyubiquitin chains and, thereby, direct them for cytosolic degradation through proteasomes (Bernasconi and Molinari, 2011).

gp78 and HRD1 are orthologues of the well-characterized yeast ER-resident ERAD E3 Hrd1p (also known as Der3p) (Fang et al., 2001; Kikkert et al., 2004). gp78 contains a complex domain structure in its C-terminal cytosolic half. In addition to its RING finger domain, it includes a coupling of ubiquitin to ER degradation (CUE) domain that can bind ubiquitin (Chen et al., 2006), and the UBE2G2-binding region (G2BR) that binds to and imparts allosteric effects on its cognate E2 (Chen et al., 2006; Li et al., 2009; Das et al., 2009). All of those domains are essential for gp78 function. Moreover, gp78 also contains a p97/valosin-containing protein (VCP)-interacting motif (VIM) that recruits the AAA-ATPase p97/VCP to ubiquitylated substrates (Ballar and Fang, 2008; Zhong et al., 2004).

gp78 is a pro-metastatic E3 ligase that mediates the degradation of the metastasis suppressor KAI1 (officially known as CD82) (Tsai et al., 2007). Also, gp78 regulates cholesterol metabolism by targeting insulin-induced gene 1 (INSIG1) to the UPS (Lee et al., 2006) (for additional gp78 substrates, please refer to the poster). HRD1 has been implicated in arthropathies including rheumatoid arthritis (Amano et al., 2003) and has substrates that include the Parkinson-disease-associated 'orphan' G-protein-coupled receptor PaelR (officially known as GPR37) (Omura et al., 2006), p53 (Yamasaki et al., 2007), and other substrates (see poster). Adding further complexity to ERAD pathways, gp78 can be targeted for degradation by autoubiquitylation and by ubiquitylation through HRD1 that – in turn – stabilizes gp78 substrates (Ballar et al., 2010; Shmueli et al., 2009). Other ER-localized E3s, including RNF5, MARCH6 and RNF139,

have also been implicated in ERAD (Morito et al., 2008; Stagg et al., 2009; Tcherpakov et al., 2009; Zavacki et al., 2009).

In mammals, the best-characterized mediator of cytosolic protein quality control is the U-box E3 CHIP (officially known as STUB1) (Cyr et al., 2002; Hohfeld et al., 2001). The U-box creates an E2 binding surface that resembles a RING finger, but lacks Zn^{2+}-coordinating sites and, instead, contains stabilizing hydrogen bonds and salt bridges (Aravind and Koonin, 2000; Hatakeyama et al., 2001; Pringa et al., 2001). CHIP contains N-terminal tetratricopeptide repeat (TPR) domains that mediate binding to HSP70, HSC70 and HSP90 chaperones (Ballinger et al., 1999; Connell et al., 2001; Nikolay et al., 2004). The CHIP U-box exists as an asymmetric homodimer that only binds one E2 molecule (Kundrat and Regan, 2010; Zhang et al., 2005).

CHIP is a chaperone-dependent E3 that modulates the switch from chaperone-assisted protein folding to UPS degradation (Connell et al., 2001; Hohfeld et al., 2001) and, thus, potentially targets any chaperone-bound protein for degradation. Of note, several CHIP substrates (e.g. α-synuclein, huntingtin, Tau) form protein aggregates that have been implicated in neurodegenerative diseases (Hatakeyama et al., 2004; Jana et al., 2005; Petrucelli et al., 2004; Shimura et al., 2004; Tetzlaff et al., 2008). Furthermore, some substrates (for example, cytochrome P450 3A4, the cystic fibrosis transmembrane conductance regulator, and others) are targeted by both ERAD E3s and CHIP (Pabarcus et al., 2009; Younger et al., 2006), which suggests cooperativity between different quality control pathways. In addition, studies in yeast have implicated other E3s in protein quality control within the cytosol as well as throughout the cell (Bengtson and Joazeiro, 2010; Eisele and Wolf, 2008; Gardner et al., 2005; Heck et al., 2010; Metzger et al., 2008; Nillegoda et al., 2010; Swanson et al., 2001); the same is likely to be true in mammalian cells.

IAP RING finger E3s and UPS in the intrinsic apoptosis pathway

Chronic protein misfolding, as well as oxidative or genotoxic stress and developmental cues, can lead to apoptosis – another process during which E3s have crucial roles (Vucic et al., 2011). In the intrinsic apoptotic pathway, stimuli trigger release of cytochrome *c* from mitochondria, leading to formation of apoptosomes. These activate caspase 9, which – in turn – activates the effector caspases 3 and 7 that mediate cell death.

Inhibitor of apoptosis proteins (IAPs) are RING E3s that prevent apoptosis at the level of caspase activation (Eckelman et al., 2006). In addition to their RING finger domains, IAPs contain baculoviral IAP repeats (BIRs) that mediate their caspase interaction. X-chromosome-linked IAP (XIAP) binds to caspases 3, 7 and 9, directly blocks their catalytic activity and, additionally, regulates their protein levels through ubiquitylation and subsequent degradation (Morizane et al., 2005; Schile et al., 2008; Suzuki et al., 2001). Cellular IAP1 (c-IAP1, officially known as BIRC2) might also promote the ubiquitylation of caspases 3 and 7, although the physiological significance of this is less clear (Choi et al., 2009).

Second mitochondrial activator of caspases (SMAC, officially known as DIABLO) is also released from mitochondria following apoptotic stimuli. SMAC prevents XIAP from interacting with and inhibiting caspases (Du et al., 2000; Verhagen et al., 2000), but SMAC itself is targeted for degradation by XIAP and cIAPs (Hu and Yang, 2003; MacFarlane et al., 2002). Further, cIAP1 can ubiquitylate XIAP, and both XIAP and c-IAP1 have autoubiquitylation activity (Cheung et al., 2008; Silke et al., 2005; Yang et al., 2000). Other RING E3s, such as SIAH1, also can target XIAP for degradation (Garrison et al., 2011), adding further complexity to the finely tuned regulation of apoptosis.

RING finger E3s in angiogenesis

Angiogenesis is a hallmark of tumor growth and progression (Hanahan and Weinberg, 2011). A key mediator of this is the hypoxia-inducible factor (HIF) family of transcription factors that induce transcription of genes involved in bioenergetics, cell growth and angiogenesis (Kaelin and Ratcliffe, 2008; Majmundar et al., 2010). Under hypoxic conditions, HIF proteins are found as transcriptionally active HIF1-α and HIF1-β (also known as HIF1A and ARNT, respectively) heterodimers that bind hypoxia response elements (HREs) upstream of target genes (Benita et al., 2009). Under normoxic conditions, HIF1-β is stable, whereas the HIF1-α proteins are degraded by the UPS (Huang et al., 1998), thereby preventing transcriptional activation. The key to oxygen-dependent regulation of HIF1-α is the von Hippel-Lindau (VHL) CRL ($CRL2^{VHL}$), which comprises the VHL tumor suppressor protein, the elongins B and C, cullin 2 and the RING protein RBX1 (Iwai et al., 1999; Kamura et al., 1999; Kibel et al., 1995; Lisztwan et al., 1999; Lonergan et al., 1998; Pause et al., 1997). VHL mediates recognition of HIF1-α proteins by binding to hydroxylated prolines, a modification that is catalyzed by the oxygen-sensing prolyl hydroxylases (PHDs) (Jaakkola et al., 2001; Ohh, 2006; Yu et al., 2001). The SIAH family of RING E3s also regulates the HIF-dependent response to hypoxia. Under hypoxic conditions, *SIAH2* transcription is induced by the HIF transcription factor. In turn, SIAH1a and SIAH2 ubiquitylate PHDs, which targets them to the UPS (Nakayama et al., 2004) and, thus, stabilizes HIF-α proteins.

Perspectives

E3s have crucial roles in cellular homeostasis and development, and frequently contribute to pathophysiological states. As our appreciation of the complexity of the ubiquitylation system increases, so do the number of questions regarding E3s, including their cellular functions, mechanisms of action and possible redundancy. Whereas humans have 600–700 putative E3s, the *Arabidopsis thaliana* genome encodes over 700 F-box proteins alone (Gagne et al., 2002), some of which have novel roles as components of SCF hormone receptors (Vierstra, 2009). Whereas, intuitively, it makes sense that sessile organisms have more varied ways of adapting to their environments, these numbers are still extraordinary.

In addition to the ligase domains discussed above, we now know that mammalian A20 zinc fingers also possess ligase activity (Mattera et al., 2006; Wertz et al., 2004). Several bacterial virulence proteins also function as ubiquitin ligases in eukaryotic host cells, despite having no sequence or structural similarity to mammalian E3s (Diao et al., 2008; Levin et al., 2010; Quezada et al., 2009). The potential for additional domains in mammals, as well as in other organisms, to have E3 activity cannot be discounted. Further complexity is added by the fact that some E3s that are believed to contain two RING finger domains (RING-in-between-RING) might actually function through a covalent thioester in a manner similar to HECTs (Wenzel et al., 2011). It is also becoming apparent that E3 domains that bind E2s in areas that are distinct from the overlapping RING or HECT and E1-binding sites – such as the G2BR of gp78, the Rad6 binding domain (R6BD) of RAD18, and the basic canyon of CUL1 (Das et al., 2009; Hibbert et al., 2011; Kleiger et al., 2009; Li et al., 2009) – might aid in the activation or regulation of ubiquitylation. Additionally, there is recent evidence that other domains within E3s, including various ubiquitin-binding domains and as-yet-uncharacterized regions, might have distinct effects on ubiquitylation. As exemplified by the discussion of E3s in this Cell Science at a Glance article, as well as other recent works (Weissman et al., 2011), autoubiquitylation and crossubiquitylation of E3s serves to regulate E3 fate and function, and is likely to be involved in many more processes than currently appreciated. The

discovery and characterization of ubiquitin ligases and their cognate substrates promises to be a fruitful and clinically important area of investigation for the foreseeable future.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.com. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.091777/-/DC1

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