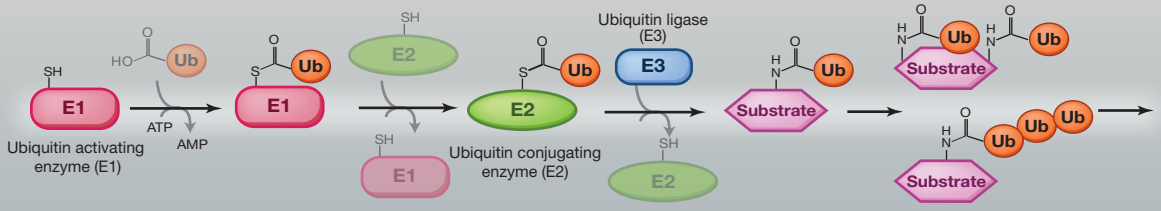
SnapShot: Types of Ubiquitin Ligases

Francesca Ester Morreale and Helen Walden
MRC Protein Phosphorylation and Ubiquitylation Unit at University of Dundee, Scotland DD1 5EH, UK

Cell

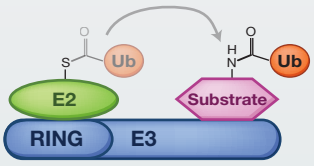
UBIQUITINATION



RING E3s

~600 in humans

Ubiquitin transfer



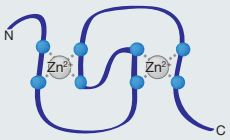
Examples of known regulation mechanisms

Neddylation (e.g., CRLs)
Phosphorylation (e.g., c-CBL, APC, Mdm2)
Small molecule: E3 interaction (e.g., TRAF-2:sphingosine-1-phosphate)

Characteristic domains

RING (Really Interesting New Gene): Zn binding domain, recruits the Ub-charged E2

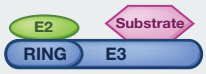
U-box: same fold as RING without Zn coordination



Types of RING E3s

Monomeric RING

Example: c-CBL



Homodimeric RING

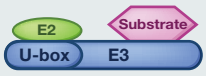
Examples: cIAP, RNF4, BIRC7, IDOL, TRIM5α

Heterodimeric RING

Examples: BRCA1–BARD1, Mdm2–MdmX, RING1B–Bmi1

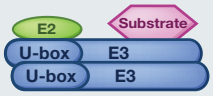
Monomeric U-box

Example: E4B

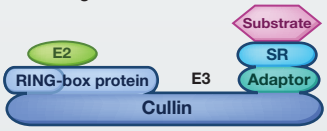


Homodimeric U-box

Examples: CHIP, Prp19



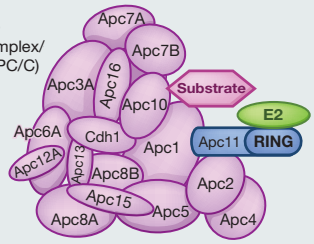
Cullin-RING ligases



Composed of:
Substrate receptor (SR) - example: F-box protein Skp2
Adaptor protein - example: Skp1
Cullin - example: Cul1
Ring-box protein (Rbx) - example: Rbx1

Other multisubunit E3s

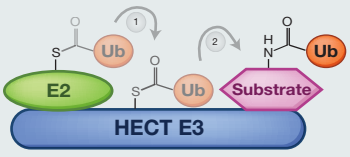
The anaphase promoting complex/ cyclosome (APC/C)



HECT E3s

~30 in humans

Ubiquitin transfer

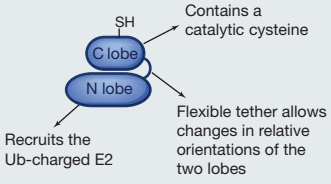


Examples of known regulation mechanisms

Autoinhibition through intramolecular interactions released by: phosphorylation (e.g., Itch), neddylation (e.g., Smurf1), protein interaction (e.g., Smurf1, Smurf2)

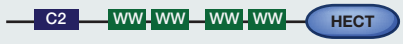
Characteristic domain

HECT (homologous to E6-AP carboxyl terminus)



HECT Families

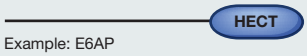
NEDD4 family



Examples: Smurf1 (2 WW motifs), Smurf2 (3 WW motifs), Itch (4 WW motifs)

HERC family

Other HECTs

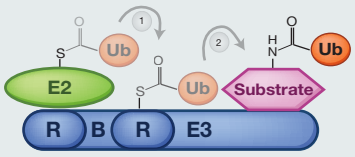


Example: E6AP

RBR E3s

~12 in humans

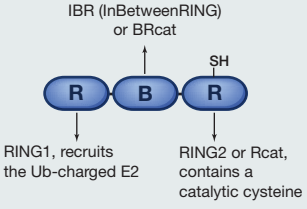
Ubiquitin transfer



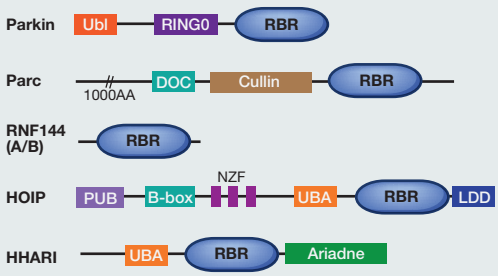
Examples of known regulation mechanisms

Autoinhibition through intramolecular interactions released by: phosphorylation/phosphoubiquitin interaction (Parkin), binding to neddylated CRLs (e.g., HHARI), protein interactions (e.g., HOIP)

Characteristic domain



Examples of RBR E3s



 DOI http://dx.doi.org/10.1016/j.cell.2016.03.003

See online version for legend and references.

SnapShot: Types of Ubiquitin Ligases

Francesca Ester Morreale and Helen Walden
MRC Protein Phosphorylation and Ubiquitylation Unit at University of Dundee, Scotland DD1 5EH, UK

Cell

Ubiquitination is a post-translational modification of proteins that is required for many cellular processes, including protein degradation by the proteasome, cell cycle progression, transcriptional regulation, DNA repair and signal transduction. Ubiquitin is covalently attached through its C terminus to the ε-amino group of lysines or (less frequently) to the N terminus of proteins with formation of an isopeptide bond.

Ubiquitination requires the sequential action of three enzymes. E1, or ubiquitin-activating enzyme, catalyzes the ATP-dependent activation of ubiquitin and formation of a thioester bond between ubiquitin C terminus and the catalytic cysteine on the E1. Ubiquitin is then transferred to a catalytic cysteine of one of the ~40 E2s (ubiquitin-conjugating enzymes) and through the E3 (ubiquitin ligase) to the substrate.

E3s are the most heterogeneous class of enzymes in the ubiquitination pathway (there are >600 E3s in humans), as they mediate substrate specificity. Currently, E3 ligases can be classified in three main types depending on the presence of characteristic domains and on the mechanism of ubiquitin transfer to the substrate protein.

RING E3s

RING E3s are the most abundant type of ubiquitin ligases. They are characterized by the presence of a zinc-binding domain called RING (Really Interesting New Gene) or by a U-box domain, which adopts the same RING fold but does not contain zinc. The RING and U-box domains are responsible for binding the ubiquitin-charged E2 and stimulating ubiquitin transfer.

RING E3s mediate a direct transfer of ubiquitin to the substrate, functioning as a scaffold to orient the ubiquitin-charged E2 with respect to the substrate protein. RING E3s can function as monomers, homodimers, or heterodimers. Homodimeric RINGs can normally bind two E2s (one per each monomer); this does not appear to be the case for heterodimeric RINGs. Similarly, U-box domains can work as monomers or homodimers.

Some RING E3s are composed by multiple subunits, such as the cullin-RING ligases (CRLs). CRLs are a highly diverse class of ubiquitin ligases characterized by several common features. They are assembled on a cullin scaffold, binding a RING-box protein at its N terminus and an adaptor protein and a substrate receptor (responsible for substrate specificity) at its C terminus.

Another important multi subunit E3 is the anaphase-promoting complex/cyclosome (APC/C), a large assembly of 19 subunits that includes a RING subunit (Apc11) and a cullin-like subunit (Apc2). RING E3s can be regulated in different ways, including neddylation, phosphorylation, and interaction with small molecules.

HECT E3s

The E3 ligases of the HECT (homologous to the E6AP carboxyl terminus) domain family catalyze ubiquitin transfer to the substrate protein through a two-step reaction: ubiquitin is first transferred to a catalytic cysteine on the E3 and then from the E3 to the substrate.

The conserved HECT domain is located at the C terminus of the proteins and is characterized by a bi-lobar architecture: the N-terminal lobe interacts with the ubiquitin-charged E2, whereas the C-terminal lobe contains the catalytic cysteine; the two lobes are tethered by a flexible hinge that allows changes in the relative orientations of the lobes during ubiquitin transfer.

While the C-terminal HECT domain is involved in the catalysis, substrate specificity is determined by the N-terminal part of the ligase. Based on their N-terminal extensions, human HECTs can be classified into three subfamilies: (1) Nedd4 family, which contains tryptophan-tryptophan (WW) motifs, (2) HERC (HECT and RCC1-like domain) family, which possesses one or more regulators of chromosome condensation 1 (RCC1)-like domains (RLDs), and (3) “other” HECTs that contain various domains.

The catalytic activity of HECT E3s is often regulated by intramolecular interactions that keep the protein in an autoinhibited state, which is released in response to various signals.

RBR E3s

In analogy with HECT E3s, RBR (RING-betweenRING-RING) E3s catalyze ubiquitin transfer through a two-step reaction where ubiquitin is first transferred to a catalytic cysteine on the E3 and then to the substrate.

The RBR name derives from the presence of two predicted RING domains (RING1 and RING2) separated by an in-between-RING domain (IBR). RING1 recruits the ubiquitin-charged E2, and RING2 domain possesses a catalytic cysteine; however, it does not conform to the canonical RING E3 structure, and it has been also called Rcat (required-for-catalysis) domain. The IBR domain adopts the same fold as the RING2 (or Rcat) domain while lacking the catalytic cysteine residue; therefore, it has been also called a BRcat (benign-catalytic) domain.

RBR E3 ligases contain additional domains that are specific to each member. Several domains are involved in intramolecular interactions that keep the protein in an autoinhibited state. Autoinhibition is released through various mechanisms, such as phosphorylation or protein-protein interactions.

REFERENCES

Berndsen, C.E., and Wolberger, C. (2014). Nat. Struct. Mol. Biol. *21*, 301–307.

Budhidarmo, R., Nakatani, Y., and Day, C.L. (2012). Trends Biochem. Sci. *37*, 58–65.

Metzger, M.B., Pruneda, J.N., Klevit, R.E., and Weissman, A.M. (2014). Biochim. Biophys. Acta *1843*, 47–60.

Primorac, I., and Musacchio, A. (2013). J. Cell Biol. *201*, 177–189.

Rotin, D., and Kumar, S. (2009). Nat. Rev. Mol. Cell Biol. *10*, 398–409.

Spratt, D.E., Walden, H., and Shaw, G.S. (2014). Biochem. J. *458*, 421–437.

Vittal, V., Stewart, M.D., Brzovic, P.S., and Klevit, R.E. (2015). J. Biol. Chem. *290*, 21244–21251.

Zimmerman, E.S., Schulman, B.A., and Zheng, N. (2010). Curr. Opin. Struct. Biol. *20*, 714–721.

  DOI http://dx.doi.org/10.1016/j.cell.2016.03.003