Hell’s BELs: Bacterial E3 Ligases That Exploit the Eukaryotic Ubiquitin Machinery

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How Do E3s Work in Eukaryotic Cells?

A common post-translational modification in eukaryotes is the covalent attachment of ubiquitin, a 76 amino acid protein, to specific proteins. Most commonly, ubiquitin is conjugated to ε-amino groups of lysine residues, and in unusual cases it can be conjugated to serine and cysteine residues or the terminal amino group of a protein. This process, referred to as ubiquitination (or ubiquitylation), can result in a variety of outcomes for a protein, depending upon how many Ub molecules are attached, whether a polyubiquitin chain is formed, and the nature of the chain. Mono-ubiquitination can result in relocalization of proteins, while most polyubiquitin chains (e.g., K48 and K11-linked chains) direct proteins for proteasomal degradation. Linkages of Ub formed using Lys 63 or by end-to-end linkages (also known as Met Ub) [1] are not directed to the proteasome and can mediate protein trafficking, scaffolding of protein complexes, or enzyme activation. Ub chains are also used for targeting invading microbes for clearance via xenophagy [2].

Ubiquitination is carried out by a series of enzymes. First, a ubiquitin activating enzyme (E1) forms a thioester with the C-terminus of Ub. The activated Ub is then transferred to one of many (~40 human) ubiquitin conjugating enzymes (E2s). Finally, the E3 enzymes (perhaps over 500 human E3s) direct the transfer of Ub to specific substrates. In eukaryotic cells there are two general classes of E3 ubiquitin ligases. The HECT (Homologous to E6-AP Carboxyl Terminus) and Ring Between Ring (RBR) domain E3s possess an invariant catalytic Cys residue that accepts Ub from a charged E2 before catalyzing transfer of Ub to substrates. Other E3s contain a Really Interesting New Gene (RING) or RING-like domain (U-box) that recruits a charged E2, as well as a domain that recruits substrates. Ub is then transferred from the E2 to the substrate, with the E3 serving primarily as a scaffold. Some RING E3s are single polypeptides, while the cullin-RING Ligases (CRLs) are modular multisubunit complexes [3]. Mammalian CRLs are nucleated by one of seven cullin family members, with a RING domain protein that binds to its C-terminus. The N-terminal region of the cullin binds specific cullin adaptor proteins that engage substrate receptor proteins; the most studied class of substrate receptors are the F-Box proteins. CRLs are subject to an additional level of control by a ubiquitin-like modifier protein, Nedd8.

E3 enzymes have two important roles. First, they recognize substrates and position them for ubiquitination. Second, E3s dictate the nature of the Ub linkage(s), which will determine the substrate’s fate. For HECT E3s, the Ub chain type is dictated by the C-terminal lobe of the HECT domain [4]. By contrast, RING-type E3s direct Ub chain type based upon the charged E2 that they recruit [5].

Successful pathogens use proteins that interfere with host cell functions that are delivered into eukaryotic host cells via specialized secretion systems and collectively referred to as “effectors.” Remarkably, although ubiquitin is restricted to eukaryotic cells, the past decade has revealed that both bacterial and viral pathogens use effectors to interfere with or manipulate the ubiquitination system [6]. This involves a large number of Bacterially encoded E3 ubiquitin Ligases (BELs). There are multiple RING-type BELs, HECT-like BELs, and even BELs that bear no resemblance to known eukaryotic ubiquitin ligases (Figure 1A).

BELs That RING

The first identification of a bacterially encoded E3 ubiquitin ligase was AvrPtoB from the plant pathogen Pseudomonas syringae pv. tomato (Pst) [7]. Plants use resistance proteins (R proteins) that recognize effectors from would-be pathogens. When R proteins engage bacterial effectors, they initiate an Effector Triggered Immune (ETI) response that prevents systemic disease [8]. AvrPtoB effectors from some P. syringae strains are recognized by the R protein Fen that initiates ETI. Although Pst AvrPtoB shared no sequence similarity to proteins of known function, the crystal structure revealed striking structural conservation with RING E3s [9]. AvrPtoB was shown to possess E3 ligase activity in vitro and to suppress ETI, demonstrating a role in pathogenesis [7]. AvrPtoB disrupts ETI by targeting Fen and Pto kinases for ubiquitination [10].

Bioinformatic approaches revealed that Legionella pneumophila encodes a number of F-box containing proteins [11]. Studies have demonstrated that these F-Box proteins function in infected cells as components of CRLs. In this case, BEL activity generates free amino acids that serve as fuel for Legionella growth inside infected cells [12]. Legionella encodes a BEL, LubX, that contains multiple U-boxes [13]. LubX targets another Legionella effector, the kinase SidB, for destruction, establishing LubX as a “metaeffector” that may act to coordinate spatiotemporal control of the effector repertoire within the host cell [14]. Thus, the targets of BELs should not necessarily be assumed to be host-encoded proteins.

Citation: Huibregtse J, Rohde JR (2014) Hell’s BELs: Bacterial E3 Ligases That Exploit the Eukaryotic Ubiquitin Machinery. PLoS Pathog 10(8): e1004255. doi:10.1371/journal.ppat.1004255

Editor: Virginia Miller, University of North Carolina at Chapel Hill School of Medicine, United States of America

Published: August 14, 2014

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Funding: Research in the Rohde laboratory is supported by Canadian Institute of Health Research (MOP-102594) and Natural Sciences and Engineering Research Council (RGPIN 386297). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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One abundant class of RING-type E3s is the NleG family, encoded in enterohaemorrhagic Escherichia coli and Citrobacter rodentium [15]. NleGs were identified using NMR structural studies. Although no sequence similarity exists, the NleGs show close structural similarity to RING-type U-box proteins. As yet, no phenotypes have been associated with nleG.
Salmonella. The IpaH family member SspH1 from Yersinia spp. [20]. The C-terminal domain is highly conserved and destroy the MAPKK Ste7 in a proteasome-dependent manner. Their N-terminal domain consists of a series of leucine rich repeat human cells are the IpaH proteins, a class of proteins that exist abundant effectors produced by Shigella. Their role in dampening host inflammation upon infection. While these effectors lack sequence homology to proteins of known function, Zhou and coworkers demonstrated that SopA functioned as a HECT-like enzyme [16]. The mechanisms of ubiquitin transfer had an absolute requirement for a catalytic Cys residue, and SopA was shown to form a Cys~Ub thioester intermediate. These data suggest a mechanism of Ub transfer similar in mechanism to that of HECT enzymes, but it is clear that there will be significant differences as well. First, the proposed substrate-binding domain is adjacent to the E2 binding site, in contrast to what has been proposed for eukaryotic HECTs [17]. Secondly, SopA and NleL interact with the same region of Ubc8 as do mammalian HECT or RING domain E3s, but differ in the precise E2 residues that are required for BEL activity [18].

**BELs That Are NELs—And More...**

*Shigella* spp. use T3SS effectors to cause shigellosis. The most abundant effectors produced by *Shigella* upon contact with human cells are the IpaH proteins, a class of proteins that exist in many gram-negative pathogens of animals and plants [19]. Their N-terminal domain consists of a series of leucine rich repeat (LRR) domains that share high similarity with YopM from *Yersinia* spp. [20]. The C-terminal domain is highly conserved among IpaH family members. Using yeast as a surrogate genetic system, IpaH98 was shown to possess E3 ubiquitin ligase activity and destroy the MAPKK Ste7 in a proteasome-dependent manner [19]. The IpaH family member SspH1 from *Salmonella* was shown to also be an E3 ligase that could ubiquitinate a known mammalian interacting protein, PKN1, in vitro [19]. Earlier studies had already shown that substrate specificity was dictated by the LRR domains [21]. The ability to ubiquitinate substrates was shown to rely on a Cys residue that is invariant among the more than 50 proteins that comprise the IpaH family [19]. Again, the IpaH family members shared no sequence similarity to proteins of known function. The crystal structure was solved independently by three groups and revealed that the catalytic domain was entirely alpha helical, had no resemblance to other E3 enzymes, and was coined the NEL domain (novel E3 ligase) [22–24]. The activity of NEL domain E3s is negatively regulated by the RING domain in the absence of their substrates, presumably to prevent premature autoubiquitination until they can productively engage their substrates [22,23,27]. The first NEL domain enzyme–substrate structure has been characterized [28]. The LRR of SspH1 binds the HR1b coiled-coil subdomain of PKN1. This report provides the first direct evidence that substrate engagement activates the catalytic activity of NELs. In this case, a straightforward “displacement model” between a linear PKN1 motif and the inhibitory residues within the NEL domain competes for binding to residues within the RING [28]. As RING domains are remarkably diverse scaffolds for protein–protein interactions, it remains to be tested if the SspH1–PKN1 paradigm will emerge as a universal mechanism for effector–substrate recognition and activation for BELs. Precise BEL–substrate interactions were recently shown to effect distinct outcomes in the Pst system. The R proteins Fen and Pto interact near the Ring domain of AvrPtoB, resulting in their ubiquitination and degradation [10]. Pto can also interact with an AvrPtoB domain distal to the Ring domain [29]. Binding at the distal domain allows Pto to evade ubiquitination by the AvrPtoB E3 ligase and to activate an ETI response. Though they do not have a ubiquitin system, bacteria encode a wide variety of E3 ubiquitin ligases that are delivered into the host cells that they infect using specialized secretion systems. A trend among BELs is that while they possess little to no sequence homology with eukaryotic E3s, they often share structural similarity. Notable exceptions are NEL domain and XL-box BELs, suggesting the idea that there are structurally related NEL domain enzymes encoded by eukaryotes. Determining the spectrum of BEL substrates in their respective hosts is an achievable goal. The identification of the eukaryotic proteins that BELs target will increase our understanding of immune functions and provide insights to help combat infection.

**Acknowledgments**

We thank Julie Ryu for assistance in producing the figure.

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