Review

Bacterial Interference of Ubiquitination and Deubiquitination

Anne Rytkönen[1] and David W. Holden[1,*]
[1] Department of Infectious Diseases, Centre for Molecular Microbiology and Infection, Imperial College London, Flowers Building, Armstrong Road, London SW7 2AZ, United Kingdom
*Correspondence: d.holden@imperial.ac.uk
DOI 10.1016/j.chom.2007.02.003

Ubiquitination and deubiquitination regulate several essential cellular processes such as protein degradation, cell-cycle progression, signaling, and DNA repair. Given the importance of these processes, it is not surprising that many microbes have developed the means to interfere with different stages of ubiquitin pathways to promote their survival and replication. This review focuses on virulence proteins of bacterial pathogens that mediate these effects and summarizes our current understanding of their actions.

Introduction

Small reversible posttranslational modifications of proteins provide a widespread mechanism for regulating a broad range of cellular processes, enabling cells to respond rapidly to changes in their environment. Such modifications include phosphorylation, acetylation, and among eukaryotes, ubiquitination and ubiquitin-like modifications such as sumoylation and neddylation.

Ubiquitin (Ub) is a small protein of 76 amino acids (Goldstein et al., 1975). It is present in all eukaryotes and is highly conserved, with only three amino acid differences between the yeast and human proteins. Ubiquitination involves covalent addition of Ub monomers to lysine residues of target proteins or of Ub itself (to form poly-Ub chains), via the involvement of at least three enzymes: a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2), and a Ub ligase (E3) (Figure 1). E1 enzymes are responsible for the ATP-dependent activation of the C terminus of Ub, which is then directed to the active site of an E2 enzyme. E3 ligases (of which there are over 1000 encoded by human genome) are a large and diverse group of proteins that have been subclassified by the presence of different motifs, including HECT, RING, and U box domains. HECT E3s transfer Ub from the E2 to a thioester linkage on the E3, and then to the substrate, while RING and U box E3s enable protein ubiquitination by acting as adaptors that bind to E2 proteins and substrates separately and facilitate direct transfer of Ub from the E2 protein to the substrate. A fourth group of E3 ligases, called the modulator of immune recognition (MIR) family, comprises membrane-bound proteins containing a specialized form of the RING finger, plant homeodomain (PHD) (Coscoy and Ganem, 2003; Ohmura-Hoshino et al., 2006).

Several RING E3s appear to act independently, but others are components of larger multiprotein complexes. A good example of the latter are the cullin RING Ub ligases. The C-terminal regions of cullin proteins interact with the E2-interacting RING protein ROC-1. The N-terminal region of cullins interact with linker or adaptor proteins such as SCF (Skp-1, Cul-1, F box), which in turn bind to the substrate. Cullins are themselves positively regulated by the binding of the small Ub-like protein (Ubl) neural precursor cell-expressed developmentally downregulated 8 (NEDD8) (RUB1 in *S. cerevisiae*).

In addition to NEDD8, other small proteins with various degrees of sequence similarity to Ub have been identified that link to proteins in an analogous manner to ubiquitination. These include small ubiquitin-like modifier 1 (SUMO-1) also known as Ubl1, Sentrin, or PIC-1 (Jentsch and Pyrowolakis, 2000; Yeh et al., 2000). Sumoylation occurs on a wide range of substrates including signaling molecules and several transcriptional regulators (Muller et al., 2001; Sapetschnig et al., 2002; Seeler and Dejean, 2001). SUMO can also act by blocking Ub attachment sites (Hoege et al., 2002). A key distinction between SUMO and Ub is the inability of SUMO to conjugate to itself (Pickart, 1997). The ability of Ub to interact with a wide range of proteins, including itself, and to form a variety of different length and linkages of chains, increases its capacity to manipulate a larger array of signals compared to Ubls. For a comprehensive review of Ub and Ub-like protein modifications, the reader is referred to a recent review (Kerscher et al., 2006).

The fate of ubiquitinated proteins depends on the combination of interacting E2 and E3 enzymes and the length and linkage of the Ub chains. Chains of four or more Ub molecules linked through lysine 48 (K48) of Ub target the modified protein for proteasomal degradation (Thrower et al., 2000). K63-linked polyubiquitin chains regulate a wide range of important cellular processes such as DNA repair, signaling, endocytosis, vesicular trafficking, and cell-cycle progression (Haglund and Dikic, 2005; Hicke and Dunn, 2003; Huang and D'Andrea, 2006). Monoubiquitination (modification with one ubiquitin molecule) controls subcellular protein localization, endocytosis, and recruitment of Ub-binding proteins (Haglund and Dikic, 2005).
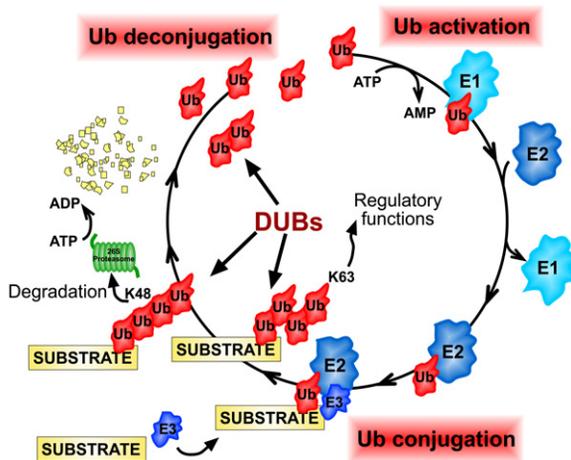
Figure 1. Schematic Figure of Ubiquitin Activation, Conjugation, and Deconjugation

E1 enzymes activate the C terminus of ubiquitin in an ATP-dependent manner, which directs ubiquitin to an E2 enzyme. E3 ligases transfer ubiquitin either from the E2 to a thioester linkage on the E3, and then to the substrate, or by acting as adaptors that bind to E2 proteins and substrates separately, and facilitate direct transfer of Ub from the E2 protein to the substrate. Chains of four or more Ub molecules linked through lysine 48 (K48) of ubiquitin target the modified protein for proteasomal degradation, whereas K63-linked polyubiquitin chains regulate several cellular processes, such as DNA repair, signaling, endocytosis, vesicular trafficking, and cell-cycle progression. Ubiquitin deconjugation is a reversible process catalyzed by deubiquitinating enzymes (DUBs). These play an important role in processing Ub precursors, proofreading Ub protein conjugates, and removing Ub from substrate proteins.

Deubiquitinating Enzymes

Protein ubiquitination is a reversible process, and Ub deconjugation, catalyzed by deubiquitinating enzymes (DUBs), plays an important role in processing Ub precursors, proofreading Ub protein conjugates, and removing Ub from target proteins (Figure 1). Major progress has been made in understanding pathways of ubiquitination, but our understanding of the functions of DUBs is much less advanced. DUBs are present in all eukaryotic cells, and approximately 100 are predicted to be encoded by the human genome. They include both metalloproteases (JAMM/MPN+) and cysteine proteases, with the majority being of the latter type. The cysteine protease DUBs have been grouped into four different classes based on their sequence and predicted activity: Ub-specific proteases (USPs, also referred to as Ub processing proteases, UBPs), Ub C-terminal hydrolases (UCHs), ovarian tumor-related proteases/Cezanne (OTUs), and Josephin domain proteases (JD) (Amerik and Hochstrasser, 2004; Nijman et al., 2005; Sulea et al., 2006). USPs represent the most diverse class, comprising proteases with two short conserved motifs, the Cys and His boxes, flanked by N- and C-terminal regions of differing length and sequence. The UCH class contains small proteins originally identified by their ability to hydrolyze small amides and esters at the C terminus of Ub. The other classes have only been recognized recently and contain only a few members. Proteases

have also been divided into families and clans according to a hierarchical classification on the basis of degree of similarity in amino acid sequence. Families of proteases that are homologous are grouped together in a clan. According to this system, cysteine proteases have been divided into 9 clans and 47 families (http://merops.sanger.ac.uk/index.htm). Several cysteine proteases that are DUBs or Ub-like protein-specific proteases (ULPs) have been classified into the clans CA and CE. In clan CA, the Cys motif is N proximal to the His box, whereas the reverse is the case in clan CE.

In contrast to many other proteases, which are translated as inactive precursors, DUBs are generally produced as active enzymes, and structural analysis has revealed that the catalytic triad only assumes the active conformation upon binding of Ub, thereby preventing nonspecific protease activity (Nijman et al., 2005). Specificity of DUBs is also likely to be determined by regions outside the catalytic core of the enzyme, and both the target and the attached Ub monomer or polymer are likely to be important for recognition by the DUB.

Bacterial Intervention

Studies on the interactions between bacteria and host cells over the last decade have revealed a remarkable diversity of cellular processes that are modulated by Gram-positive and Gram-negative pathogens. Several Gram-negative bacterial pathogens use type three secretion (T3S) systems to deliver effector proteins into host cells. Components of the T3S apparatus (which resembles a needle-like structure in both form and function; Galan and Wolf-Watz, 2006) are conserved in different pathogens; however, each pathogen is endowed with a unique repertoire of effectors that contribute to the disease processes caused by that pathogen. These effectors interfere with the actin, microtubule, and intermediate filament cytoskeletons; microtubule motors; specific stages of the endocytic and secretory pathways; and a host of signaling pathways, affecting, for example, transcription, translation, cell division, cell migration, and immune responses. Given the importance of ubiquitination and deubiquitination in the regulation of many cellular processes, it is not surprising that pathogenic bacteria have developed the means to interfere extensively with different stages of Ub pathways. In this review we focus on the bacterial virulence proteins that mediate these effects (summarized in Table 1), and what is known of the mechanisms by which they interfere with Ub pathways.

Yersinia YopJ/P

The first bacterial protein shown to have DUB activity was the *Yersinia* T3S effector YopJ/P (referred to as YopJ in *Yersinia pseudotuberculosis* and YopP in *Yersinia enterocolitica*) (Figure 2). YopJ is present in all three *Yersinia* spp. pathogenic for humans; these bacteria are mainly extracellular pathogens causing both enteric diseases (*Y. pseudotuberculosis* and *Y. enterocolitica*) and plague (*Y. pestis*). YopJ plays an important role in inhibiting the inflammatory response and inducing apoptosis in

Table 1. Summary of Bacterial Effectors Directly Targeting Ub Pathways

Pathogen	Virulence Protein	Biochemical Function	Target(s)	Physiological Effect(s)	Reference
C. trachomatis	ChlaDub1	DUB and deneddylator	unknown	unknown	Misaghi et al., 2006
	ChlaDub2	DUB and deneddylator	unknown	unknown	Misaghi et al., 2006
P. syringae	AvrPtoB	E3 ligase	unknown	suppression of plant immunity	Janjusevic et al., 2006
S. Typhimurium	SseL	DUB	unknown	cytotoxicity	Rytkönen et al., 2007
	SopA	E3 ligase		induction of PMN trans-epithelial migration	Zhang et al., 2006
S. flexneri	OspG	kinase	Ub E2 molecules	suppression of host innate response	Kim et al., 2005
	IpaH9.8	E3 ligase	MAPKK Ste7 in yeast	suppression of inflammatory response	Rohde et al., 2007; Okuda et al., 2005
Yersinia	YopJ/YopP	DUB/acetyl-transferase	unknown/MKKs and IKKs	suppression of MAPK and NF-κB pathway, induction of apoptosis	Zhou et al., 2005; Mukherjee et al., 2006; Mittal et al., 2006

PMN, polymorphonuclear leukocytes.

macrophages. By interfering with the NF-κB pathway through inhibition of MAPK (mitogen-activated protein kinase) kinases and IKKβ, YopJ suppresses proinflammatory signaling via TNF and IL-8 production, as well as inducing apoptosis (Boland and Cornelis, 1998; Mills et al., 1997; Monack et al., 1997; Orth et al., 1999; Palmer et al., 1998; Yoon et al., 2003; Zhou et al., 2004). YopJ is a member of clan CE, family 55, and its substrate was originally thought to be SUMO-1, since expression of YopJ in mammalian cells decreased the cellular concentration of SUMO-1-conjugated proteins (Orth et al., 2000). However, a subsequent study showed that purified YopJ cleaves Ub-amino methyl coumarin (Ub-AMC) (a fluorogenic Ub conjugate) but not SUMO-1-AMC in vitro (Zhou et al., 2005). Furthermore, infection of macrophages with a Y. pseudotuberculosis strain expressing a mutant version of YopJ in which the catalytic cysteine was replaced by alanine resulted in the accumulation of ubiquitinated IκBα in the macrophage cytosol (Zhou et al., 2005). Normally, activation of the NF-κB pathway leads to phosphorylation, ubiquitination, and proteasomal degradation of IκBα, which allows the translocation of NF-κB to the nucleus, where it regulates transcription of genes involved in proinflammatory signaling. Translocated YopJ was therefore proposed to act as a DUB for ubiquitinated IκBα, thereby preventing its degradation. However, YopJ is evidently not able to hydrolyze ubiquitinated IκBα in vitro (Collier-Hyams et al., 2002). Furthermore, it has been shown recently by two groups that YopJ can function as an acetyltransferase in vitro by transferring acetyl groups to serine and threonine residues in the activation loop of MAPKK6, effectively preventing their phosphorylation and hence kinase activation (Bliska, 2006; Mittal et al., 2006; Mukherjee et al., 2006). It is important to know if YopJ can cause similar modifications in vivo, since it is not immediately obvious how a loss of acetyltransferase activity on MAPKK6 would result in an accumulation of ubiquitinated IκBα in infected cells (Zhou et al., 2005). However, these studies raise the possibility that YopJ is a bifunctional enzyme that acetylates one or more substrate and deubiquitinates another. Such relaxed substrate specificity of a bacterial enzyme would not be unprecedented: E. coli TAP is a multifunctional enzyme that has both protease and deacetylating activities (Lee et al., 2006).

Salmonella

Salmonella enterica serovar Typhimurium is an invasive pathogen that colonizes the small intestine, and in the murine model of typhoid it penetrates the epithelium and transits from Peyer's patches and mesenteric lymph nodes to the spleen and liver, where it replicates in macrophages. Following invasion or phagocytosis by macrophages, the vast majority of intracellular S. enterica remain in a membrane-bound compartment, the Salmonella-containing vacuole (SCV), which the bacteria modify to provide an environment favorable for replication. To invade host cells, Salmonella uses a T3S system encoded by the Salmonella pathogenicity island 1 (SPI-1). This system translocates several effector proteins across the host-cell plasma membrane. Some of these remodel the actin cytoskeleton to cause localized membrane ruffling and bacterial uptake. Thereafter, another set of effectors is translocated across the vacuolar membrane. This is accomplished by the T3S system encoded by SPI-2, which translocates over 20 effectors, detectable approximately 4 hr following entry of bacteria. Together, these effector proteins enable bacterial replication in a variety of
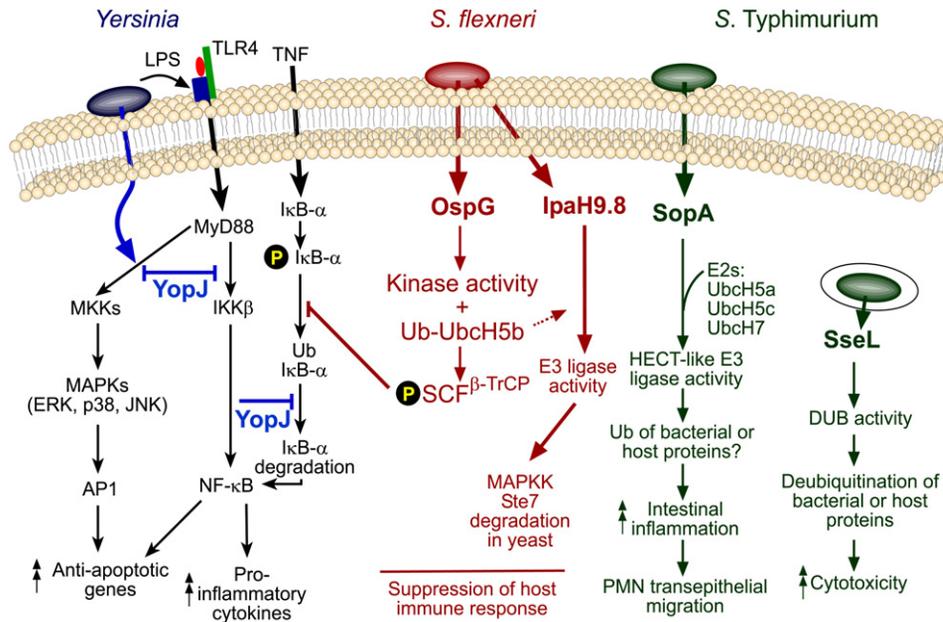
Figure 2. Examples of Interference of Mammalian Ub Pathways by Bacterial Pathogens

YopJ/P, a *Yersinia* T3S effector protein with DUB and acetyltransferase activity, is translocated into host cells, where it interferes with the NF-κB pathway through inhibition of MKKs (mitogen-activated protein kinase kinases) and IKKβ and possibly by deubiquitinating ubiquitinated IκB-α. YopJ plays an important role in inhibiting the inflammatory response and induces apoptosis in macrophages. *Shigella* also inhibits host immune responses. Following translocation into host cells, OspG acts as a kinase and blocks TNF-induced IκBα degradation, conceivably by phosphorylating a component of SCF$^{β-TrCP}$ complex. IpaH9.8, another *Shigella* effector, functions as an E3 Ub ligase that interferes with the pheromone response signaling pathway in yeast by ubiquitinating the MAPKK Ste7. To facilitate invasion and intracellular survival, *Salmonella* translocates two sets of effector proteins into the host cells. The *Salmonella* pathogenicity island 1 (SPI-1) T3S system translocates effector proteins across the host-cell plasma membrane. Several of these effectors are ubiquitinated following translocation, but one, SopA, functions as a HECT-like E3 Ub ligase that preferentially uses the inflammation-associated E2s UbcH5a, UbcH5c, and UbcH7 for ubiquitination in vitro. SopA induces a host inflammatory response by promoting polymorphonuclear leukocyte transepithelial migration. Following uptake, *Salmonella* translocates another set of effectors across the *Salmonella*-containing vacuole via the SPI-2 T3S system. SseL is translocated approximately 6 hr postuptake and acts as a DUB in vivo to trigger a delayed cytotoxic effect in macrophages.

host-cell types and also induce a delayed cytotoxicity in macrophages (Kuhle and Hensel, 2004; Waterman and Holden, 2003).

Salmonellae that are both pathogenic and nonpathogenic for humans interfere with ubiquitination pathways. Whereas pathogenic *Salmonella* elicits an NF-κB-dependent proinflammatory response upon colonization of human intestinal epithelial cells in vitro, nonpathogenic strains are capable of suppressing this response. The inhibition does not affect the phosphorylation of IκBα but somehow affects its subsequent ubiquitination, and thereby prevents its degradation (Neish et al., 2000). The inhibition appears to be specific to the E3-SCF$^{β-TrCP}$ substrates IκBα and β-catenin and caused by a rapid loss of neddylated Cullin-1, which is a subunit of the E3-SCF complex necessary for ubiquitination of IκBα and subsequent activation of NF-κB (Collier-Hyams et al., 2005). The *Salmonella* molecule(s) responsible and the mechanisms involved in this interesting activity are not yet known.

AvrA is predicted to be a clan CE protein translocated by the SPI-1 T3S system in a subset of strains of *S. enterica* (Hardt and Galan, 1997). AvrA has 56% identity to YopJ and also inhibits the NF-κB pathway. However, this seems to occur at a different stage than YopJ, because in transfected cells YopJ had little effect on

NF-κB-dependent reporter gene activity when cells were stimulated with IKK-β, whereas AvrA effectively inhibited reporter gene activity (Collier-Hyams et al., 2002). However, like YopJ, AvrA contributes to apoptosis of host cells (Collier-Hyams et al., 2002). While the inhibitory activity of AvrA on the NF-κB pathway was shown to be dependent on a putative catalytic cysteine, it has yet to be established if AvrA is a DUB.

Several other effectors involved in *Salmonella* invasion are either ubiquitinated or modulate ubiquitination following their translocation to the host cells. SopE is a guanine nucleotide exchange factor for Rac1 that stimulates the actin polymerization required for membrane ruffling and bacterial uptake (Patel and Galan, 2006). SptP, which is codelivered into host cells in similar quantities to SopE, acts as a GTPase-activating protein and counteracts the activity of SopE (Fu and Galan, 1999). SopE is rapidly ubiquitinated and degraded, while the half-life of SptP is much longer. Domain-swapping experiments revealed that differential degradation kinetics is controlled by information in the N-terminal secretion and translocation domains of these proteins. The differential half-life of these proteins therefore accounts for the transient nature of the ruffling response induced by *Salmonella* (Kubori and Galan, 2003).

SopA is another SPI-1 effector that has an important role in the induction of host inflammatory responses by promoting polymorphonuclear (PMN) leukocyte transepithelial migration (Wood et al., 2000) (Figure 2). In epithelial cells, translocated SopA is ubiquitinated by an E3 ligase, HsRMA1, and subsequently degraded. While the majority of *S. enterica* serovar Typhimurium (*S.* Typhimurium) bacteria remain within membrane-bound vacuoles, a small proportion escape into the epithelial cell cytosol, where they replicate. Ubiquitinated SopA appears to have a minor role in promoting vacuolar escape, since mutation of *sopA* or RNAi of HsRMA1 reduced the numbers of bacteria that escaped their vacuoles (Zhang et al., 2005). In macrophages, cytosolic bacteria fail to replicate (Beuzon et al., 2002) and become surrounded by polyubiquitinated proteins and proteasomes, which might contribute to bacterial growth suppression (Perrin et al., 2004). Remarkably, recent work has shown that SopA is itself a HECT-like E3 Ub ligase (Zhang et al., 2006). Consistent with a regulatory role in inflammation, SopA preferentially used the inflammation-associated E2s UbcH5a, UbcH5c, and UbcH7 for ubiquitination in vitro, and a *Salmonella* strain expressing an E3 ligase-dead point mutant version of SopA induced less PMN transepithelial migration in vitro (Zhang et al., 2006). SopA could ubiquitinate bacterially derived or host proteins (or both), and identification of its physiological target(s) is now crucial in understanding its role in inflammation and enteritis.

SigD/SopB is a fourth SPI-1 effector that is ubiquitinated upon translocation to the host cell. This inositol phosphatase localizes to the surface of the nascent SCV, where it persists for several hours (Marcus et al., 2002). It acts by retaining high levels of phosphatidylinositol-3-phosphate [PtdIns(3)P] in the SCV membrane and has important roles in bacterial replication (Galan and Collmer, 1999; Hernandez et al., 2004) and suppression of apoptosis in epithelial cells by activation of Akt (Knodler et al., 2005), and membrane fission during bacterial invasion (Terebiznik et al., 2002). By 2 hr postinvasion, ubiquitin-conjugated SigD/SopB can be detected, but curiously this does not appear to lead to its degradation by the proteasome (Marcus et al., 2002), and the functional significance of its ubiquitination (if any) is not understood.

Interference with ubiquitination by *Salmonella* continues after the expression of the SPI-2 T3S system. SseL is a newly characterized effector of cysteine protease clan CE whose translocation onto the vacuolar membrane can be detected in epithelial cells and macrophages after approximately 6 hr following uptake of bacteria (Rytkönen et al., 2007) (Figure 2). A yeast two-hybrid screen with SseL and a HeLa cell cDNA library revealed that SseL interacts directly with Ub. A purified GST-SseL fusion protein specifically cleaved ubiquitin substrates, and infection of epithelial cells and macrophages with *S.* Typhimurium *sseL* mutant strains led to the accumulation of ubiquitin-modified proteins, indicating that SseL functions as a DUB in vivo. However, unlike YopJ, SseL did not influence cytokine production or interfere with activation or degradation of IκBα upon infection of macrophages.

sseL mutant strains did not display an intracellular replication defect but were defective for a SPI-2-dependent late-stage cytotoxic effect on macrophages and were attenuated for virulence in the systemic phase of infection in mice (Rytkönen et al., 2007). This shows that ubiquitinated proteins of host or bacterial origin accumulate on (or in close proximity to) the SCV membrane, and these are deubiquitinated by SseL. How this leads to cytotoxicity remains to be established, but the study provides evidence that the cytotoxic activity of *Salmonella* is an important component of its virulence. It is tempting to speculate that the deubiquitinating activity of SseL might function to counteract ubiquitination and degradation of other translocated bacterial effectors by the host-cell machinery. However, this seems unlikely for two reasons. First, an important collective function of SPI-2 T3S system effectors is to enable intracellular growth (Waterman and Holden, 2003) and if SseL acted by protecting these effectors then an *sseL* mutant would be predicted to have an intracellular replication defect. Secondly, SseL has a clear preference for K63-linked Ub chains (Rytkönen et al., 2007), indicating that it is likely to be involved in signaling events rather than interfering directly with protein degradation.

Shigella

Shigella flexneri is an important cause of human bacillary dysentery. The morphological features of *Shigella* entry into epithelial cells are similar to those induced by *S.* Typhimurium, and although both require T3S systems, the effector protein repertoire and infection process following invasion are distinct. *S. flexneri* invades the colonic epithelium initially through M cells of Peyer's patches. Bacteria then interact with macrophages in the lymphoid follicle, and enterocytes via their basolateral membranes. These interactions result in a strong proinflammatory response that exacerbates infection of the intestinal epithelium. However, it has become clear that stimulation of proinflammatory responses is subsequently modulated by the action of several bacterial effectors (Arbibe et al., 2007; Kim et al., 2005). OspG is one of approximately 20 effectors delivered to the host cells by the *Shigella* T3S system. A yeast two-hybrid screen showed that OspG interacts with ubiquitinylated E2 molecules, including UbcH5b (Figure 2). OspG was shown to have kinase activity but evidently does not phosphorylate E2 proteins. Nevertheless, OspG can block TNF-induced IκBα degradation, a process that is dependent on UbcH5b (Kim et al., 2005). This effect requires a lysine residue important for kinase activity of OspG and suggests that OspG acts at the step of phospho-IκBα ubiquitination. Possibly, OspG acts by phosphorylating a component of $SCF^{\beta-TrCP}$ in the context of OspG-UbcH5b. The biochemical evidence for dampening of the inflammatory response is supported by the phenotype of an *ospG* mutant, which induced a much stronger inflammatory response during infection of rabbit ileal loops (Kim et al., 2005).

IpaH9.8 is another effector delivered by *Shigella* into host cells, where it translocates to the nucleus (Haraga

and Miller, 2003; Toyotome et al., 2001). Recently it has been shown that IpaH9.8 is an E3 Ub ligase (Rohde et al., 2007) (Figure 2). Its biochemical characterization was achieved by using *Saccharomyces* both as a host for an initial phenotypic screen, and then to identify its target and biochemical function. IpaH9.8 was shown to interfere with the pheromone response signaling pathway by ubiquitinating the MAPKK Ste7, which leads to its proteasome-dependent destruction (Rohde et al., 2007). Work from another group has shown that, in mammalian cells, IpaH9.8 interacts with U2AF^{35}—a component of an essential pre-mRNA splicing factor complex (Okuda et al., 2005). Deletion of *ipaH9.8* resulted in increased proinflammatory cytokine production and, consistent with the hypothesis that IpaH9.8 might interfere with the function of U2AF^{35}, reduction of U2AF^{35} expression by siRNA-mediated knockdown caused a decrease in the expression of these cytokines (Okuda et al., 2005). It would be interesting to know if IpaH9.8 can ubiquitinate U2AF^{35}, which might help to reconcile the results from these two studies. The finding that IpaH9.8 is an E3 ligase has broader implications, since homologous proteins with similar domain organization and containing a critical cysteine residue are found not only in *Shigella* but also in several other bacterial pathogens. These proteins include *Salmonella* SspH1, an effector of both the SPI-1 and SPI-2 T3S systems. Purified SspH1 was shown to have E3 ligase activity on PKN1 (Rohde et al., 2007), a host-cell kinase previously shown to interact with SspH1 (Haraga and Miller, 2006).

Chlamydia

The availability of ubiquitin-based probes has provided useful reagents to identify new DUBs from mammalian cells and viruses (Galardy et al., 2005; Ovaa et al., 2005). Recently, this approach was used to identify two DUBs from *Chlamydia trachomatis* (Misaghi et al., 2006). Lysates of infected HeLa cells were incubated with an epitope-tagged Ub-vinylmethylester conjugate, which forms a covalent adduct with the active site cysteine of DUBs (Borodovsky et al., 2001). Following immunoprecipitation, an interacting protein was fractionated by SDS-PAGE and identified by mass spectrometry. Genome sequence analysis revealed that the gene encoding this DUB is flanked by a homolog. Both corresponding proteins (named *Chla*Dub1 and *Chla*Dub2) were shown to have deubiquitinating and deneddylating activities. Although *Chla*Dub1 is clearly translocated into host cells by bacteria, its localization, biochemical target(s), and possible role during infection remain to be established.

Escherichia coli

CNF1 toxin is produced by uropathogenic *E. coli*, and related toxins have been found in *Y. pseudotuberculosis* and *Bordetella pertussis*. The toxin is taken up by mammalian cells by endocytosis and then passes into the cytosol by translocation through the endosomal membrane (Boquet, 2001). CNF1 functions by deamidating a specific glutamine residue of Rho GTPases to glutamate. This prevents endogenous or induced GTPase activity of these molecu-

lar switches, effectively locking them into a permanently active state. However, this effect is only transient, as the host cell responds by depleting the modified GTPases by ubiquitination and proteasome-mediated degradation (Doye et al., 2002). Hence, although the immediate effect of CNF1 deamidation of Rho proteins is their activation, the long-term consequence is ubiquitination and degradation. In the case of RhoA, this is carried out by an E3 ligase called Smurf1 (Boyer et al., 2006).

Listeria

Listeria monocytogenes is a Gram-positive bacterial pathogen that interacts with host-cell surface proteins to trigger signaling pathways leading to bacterial uptake. One such pathway involves a bacterial cell surface-localized protein, InlB, and the growth factor receptor Met (Shen et al., 2000). Interaction between Met and its ligand normally triggers the recruitment of the Ub ligase Cbl, which ubiquitinates Met and leads to its endocytosis. It was found recently that InlB can mediate the same process, and furthermore that the Ub-dependent endocytosis machinery is required for internalization of bacteria (Veiga and Cossart, 2005). While the interaction between InlB and ubiquitination machinery is indirect, this discovery reveals another means by which bacterial pathogens harness host Ub-dependent pathways to promote their virulence. Following uptake into host cells, *L. monocytogenes* escapes its vacuole and begins to replicate and spread in the host-cell cytosol. This is dependent on the action of the secreted cytolysin listeriolysin O (LLO). LLO is potentially damaging to the host-cell plasma membrane, and to ensure that bacteria remain intracellular, the activity of LLO must be restricted to the vacuolar membrane. By mechanisms that are still unclear, LLO is rapidly ubiquitinated and degraded by the proteasome machinery (Schnupf et al., 2006).

Plant Pathogens

Bacterial plant pathogens also use specialized secretion systems to deliver effector proteins into host cells. As with animal pathogens, the processes of activation, conjugation, and deconjugation of Ub and Ubls all appear to be targeted by bacterial effector proteins. VirF of *Agrobacterium* is one of several proteins involved in transfer of T-DNA from this bacterium into plant cells. It localizes to the plant cell nucleus, where it causes the degradation of a plant protein VIP1. This is thought to be mediated by an F box motif in VirF that binds the plant homolog of Skp1, a component of SCF in some E3 ligase complexes (Tzfira et al., 2004). AvrPtoB is a T3S effector translocated into tomato cells by *Pseudomonas syringae*, where it inhibits hypersensitivity—a plant immune response involving rapid localized host-cell death at the site of infection. The amino acid sequence of AvrPtoB provided no information as to its function, but the crystal structure of the C-terminal domain of AvrPtoB revealed striking similarity to U box and RING finger components of E3 ligases (Janjusevic et al., 2006). Purified AvrPtoB was shown to have E3 ligase activity in vitro, and putative E2 binding site

residues were shown to be critical for suppression of the plant hypersensitive response (Janjusevic et al., 2006). Presumably, AvrPtoB recruits E2 partners and substrates and transfers Ub to host-cell proteins(s) that positively regulate the plant defense response. However, the cellular substrate(s) of this activity have not yet been identified. *Xanthomonas campestris* is a related pathogen of solanaceous plants; one of its T3S system effectors, XopD, has been characterized as a cysteine protease of clan CE, family 48. This effector localizes to the plant cell nucleus, where it cleaves (as yet uncharacterized) sumoylated proteins (Hotson et al., 2003). XopD appears to have a strict preference for plant SUMOs and was recently used to obtain insight into substrate specificity of ULPs (Chosed et al., 2007). Several other bacterial plant pathogens and symbionts are predicted to contain proteins that have been classified as members of clan CE, family 48 proteases. These include *Acidovorax avenae*, *Bradyrhizobium japonicum*, *Mesorhizobium loti*, and *Rhizobium leguminosarum* (http://merops.sanger.ac.uk/index.htm).

Viral Intervention

Although this review focuses on bacteria, several viruses are also known to interfere with different steps in the ubiquitin pathway. Some encode proteins that modify cullin-like RING Ub ligases by interacting with substrate adaptors, redirecting the Ub ligase complex to new targets, or protecting cellular proteins from ubiquitination (Barry and Fruh, 2006). Both the Herpes and Poxvirus families encode proteins with E3 ligase activity. These E3 ligases promote ubiquitination of MHC-1, B7.2, ICAM-1, and CD4, resulting in removal of these cell surface molecules and obstruction of host immune responses (Coscoy et al., 2001; Duncan et al., 2006; Hewitt et al., 2002; Mansouri et al., 2003). Other viruses produce E3 ligases that interfere with apoptosis and interferon (IFN) production (Halford et al., 2006; Melroe et al., 2004; Taylor and Barry, 2006). Recently, it has become clear that some viruses, including herpesvirus, cytomegalovirus, adenovirus, and coronaviruses also encode DUBs; however, the targets for most of these DUBs have not yet been identified (Kattenhorn et al., 2005; Balakirev et al., 2002; Barretto et al., 2005; Sulea et al., 2006; Wang et al., 2006).

Conclusions

From the work reviewed above and elsewhere (Boyer and Lemichez, 2004; Angot et al., 2007), it is clear that interference of ubiquitination and deubiquitination plays an important role in the virulence of several bacterial pathogens and frequently influences host immune responses. Although the current list of bacterial effectors involved in these processes is relatively short, they would appear to fall into three general categories: those that act directly on pathways by "mimicking" host-cell proteins (e.g., E3 ligases and DUBs), those that act indirectly (e.g., *E. coli* CNF1 and *Shigella* OspG), and those that are themselves subject to ubiquitination in ways that influence bacterial virulence (e.g., *Salmonella* SopE). Much of our (albeit limited) understanding of this aspect of bacterial virulence

has been gained in the last few years, and it therefore seems likely that the number of bacterial proteins that either mimic or modulate host-cell proteins involved in Ub pathways will increase considerably in the future. Of the bacterial DUBs that have been identified to date, none have the same architecture as the "classic" eukaryotic DUBs (clan CA); they are all members of clan CE proteases. As our knowledge of the clan organization of DUBs and ULPs increases (Hotson and Mudgett, 2004), it will become easier to identify putative new Ub- or ULP-specific proteases among both plant and animal pathogens. However, identifying new modifiers of the Ub conjugation machinery is unlikely to be straightforward, since the bacterial E3 ligases identified to date do not display striking amino acid sequence similarities, either between themselves or with their mammalian counterparts. A major problem is that the targets of most of these bacterial effectors are currently unknown, and identifying them can be fraught with difficulty: many of these effectors are very potent, are produced transiently, and localize to specific regions of the infected cell. Specificity could therefore be achieved by precise spatiotemporal control of effector delivery rather than being an intrinsic property of the effectors. The controversial literature regarding the function of YopJ emphasizes the need for careful biochemical analysis of putative cysteine proteases to reveal their physiological functions in vivo. Ideally, this involves the study of the protein in the natural context of infection, as opposed to analysis following its overexpression either in vitro or in vivo, but obviously this is not feasible in many instances. Notwithstanding these concerns, the growing literature on bacterial manipulation of Ub pathways reflects a very important and widespread aspect of bacterial pathogenesis, and future research in this area will provide exciting new insights into the mechanisms underlying bacterial virulence.

REFERENCES

Amerik, A.Y., and Hochstrasser, M. (2004). Mechanism and function of deubiquitinating enzymes. Biochim. Biophys. Acta *1695*, 189–207.

Angot, A., Vergunst, A., Genin, S., and Peeters, N. (2007). Exploitation of eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III and type IV secretion systems. PLoS Pathog. *3*, e3. 10.1371/journal.ppat.0030003.

Arbibe, L., Kim, D.W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C., and Sansonetti, P.J. (2007). An injected bacterial effector targets chromatin access for transcription factor NF-kappaB to alter transcription of host genes involved in immune responses. Nat. Immunol. *8*, 47–56.

Balakirev, M.Y., Jaquinod, M., Haas, A.L., and Chroboczek, J. (2002). Deubiquitinating function of adenovirus proteinase. J. Virol. *76*, 6323–6331.

Barretto, N., Jukneliene, D., Ratia, K., Chen, Z., Mesecar, A.D., and Baker, S.C. (2005). The papain-like protease of severe acute respiratory syndrome coronavirus has deubiquitinating activity. J. Virol. *79*, 15189–15198.

Barry, M., and Fruh, K. (2006). Viral modulators of cullin RING ubiquitin ligases: Culling the host defense. Sci. STKE *2006*, pe21.

Beuzon, C.R., Salcedo, S.P., and Holden, D.W. (2002). Growth and killing of a Salmonella enterica serovar Typhimurium sifA mutant strain in the cytosol of different host cell lines. Microbiol. *148*, 2705–2715.

Bliska, J.B. (2006). Yersinia inhibits host signaling by acetylating MAPK kinases. ACS Chem. Biol. *1*, 349–351.

Boland, A., and Cornelis, G.R. (1998). Role of YopP in suppression of tumor necrosis factor alpha release by macrophages during Yersinia infection. Infect. Immun. *66*, 1878–1884.

Boquet, P. (2001). The cytotoxic necrotizing factor 1 (CNF1) from Escherichia coli. Toxicon *39*, 1673–1680.

Borodovsky, A., Kessler, B.M., Casagrande, R., Overkleeft, H.S., Wilkinson, K.D., and Ploegh, H.L. (2001). A novel active site-directed probe specific for deubiquitylating enzymes reveals proteasome association of USP14. EMBO J. *20*, 5187–5196.

Boyer, L., and Lemichez, E. (2004). Targeting of host-cell ubiquitin and ubiquitin-like pathways by bacterial factors. Nat. Rev. Microbiol. *2*, 779–788.

Boyer, L., Turchi, L., Desnues, B., Doye, A., Ponzio, G., Mege, J.L., Yamashita, M., Zhang, Y.E., Bertoglio, J., Flatau, G., et al. (2006). CNF1-induced ubiquitylation and proteasome destruction of activated RhoA is impaired in Smurf1−/− cells. Mol. Biol. Cell *17*, 2489–2497.

Chosed, R., Tomchick, D.R., Brautigam, C.A., Mukherjee, S., Negi, V.S., Machius, M., and Orth, K. (2007). Structural analysis of Xanthomonas XopD provides insights into substrate specificity of ULPs. J. Biol. Chem., in press.

Collier-Hyams, L.S., Zeng, H., Sun, J., Tomlinson, A.D., Bao, Z.Q., Chen, H., Madara, J.L., Orth, K., and Neish, A.S. (2002). Cutting edge: Salmonella AvrA effector inhibits the key proinflammatory, anti-apoptotic NF-kappa B pathway. J. Immunol. *169*, 2846–2850.

Collier-Hyams, L.S., Sloane, V., Batten, B.C., and Neish, A.S. (2005). Cutting edge: Bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. J. Immunol. *175*, 4194–4198.

Coscoy, L., and Ganem, D. (2003). PHD domains and E3 ubiquitin ligases: Viruses make the connection. Trends Cell Biol. *13*, 7–12.

Coscoy, L., Sanchez, D.J., and Ganem, D. (2001). A novel class of herpesvirus-encoded membrane-bound E3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition. J. Cell Biol. *155*, 1265–1273.

Doye, A., Mettouchi, A., Bossis, G., Clement, R., Buisson-Touati, C., Flatau, G., Gagnoux, L., Piechaczyk, M., Boquet, P., and Lemichez, E. (2002). CNF1 exploits the ubiquitin-proteasome machinery to restrict Rho GTPase activation for bacterial host cell invasion. Cell *111*, 553–564.

Duncan, L.M., Piper, S., Dodd, R.B., Saville, M.K., Sanderson, C.M., Luzio, J.P., and Lehner, P.J. (2006). Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. EMBO J. *25*, 1635–1645.

Fu, Y., and Galan, J.E. (1999). A salmonella protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. Nature *401*, 293–297.

Galan, J.E., and Collmer, A. (1999). Type III secretion machines: Bacterial devices for protein delivery into host cells. Science *284*, 1322–1328.

Galan, J.E., and Wolf-Watz, H. (2006). Protein delivery into eukaryotic cells by type III secretion machines. Nature *444*, 567–573.

Galardy, P., Ploegh, H.L., and Ovaa, H. (2005). Mechanism-based proteomics tools based on ubiquitin and ubiquitin-like proteins: Crystal-lography, activity profiling, and protease identification. Methods Enzymol. *399*, 120–131.

Goldstein, G., Scheid, M., Hammerling, U., Schlesinger, D.H., Niall, H.D., and Boyse, E.A. (1975). Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proc. Natl. Acad. Sci. USA *72*, 11–15.

Haglund, K., and Dikic, I. (2005). Ubiquitylation and cell signaling. EMBO J. *24*, 3353–3359.

Halford, W.P., Weisend, C., Grace, J., Soboleski, M., Carr, D.J., Balliet, J.W., Imai, Y., Margolis, T.P., and Gebhardt, B.M. (2006). ICP0 antagonizes Stat 1-dependent repression of herpes simplex virus: Implications for the regulation of viral latency. Virol. J. *3*, 44.

Haraga, A., and Miller, S.I. (2003). A Salmonella enterica serovar typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. Infect. Immun. *71*, 4052–4058.

Haraga, A., and Miller, S.I. (2006). A Salmonella type III secretion effector interacts with the mammalian serine/threonine protein kinase PKN1. Cell. Microbiol. *8*, 837–846.

Hardt, W.D., and Galan, J.E. (1997). A secreted Salmonella protein with homology to an avirulence determinant of plant pathogenic bacteria. Proc. Natl. Acad. Sci. USA *94*, 9887–9892.

Hernandez, L.D., Hueffer, K., Wenk, M.R., and Galan, J.E. (2004). Salmonella modulates vesicular traffic by altering phosphoinositide metabolism. Science *304*, 1805–1807.

Hewitt, E.W., Duncan, L., Mufti, D., Baker, J., Stevenson, P.G., and Lehner, P.J. (2002). Ubiquitylation of MHC class I by the K3 viral protein signals internalization and TSG101-dependent degradation. EMBO J. *21*, 2418–2429.

Hicke, L., and Dunn, R. (2003). Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu. Rev. Cell Dev. Biol. *19*, 141–172.

Hoege, C., Pfander, B., Moldovan, G.L., Pyrowolakis, G., and Jentsch, S. (2002). RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature *419*, 135–141.

Hotson, A., and Mudgett, M.B. (2004). Cysteine proteases in phytopathogenic bacteria: Identification of plant targets and activation of innate immunity. Curr. Opin. Plant Biol. *7*, 384–390.

Hotson, A., Chosed, R., Shu, H., Orth, K., and Mudgett, M.B. (2003). Xanthomonas type III effector XopD targets SUMO-conjugated proteins in planta. Mol. Microbiol. *50*, 377–389.

Huang, T.T., and D'Andrea, A.D. (2006). Regulation of DNA repair by ubiquitylation. Nat. Rev. Mol. Cell Biol. *7*, 323–334.

Janjusevic, R., Abramovitch, R.B., Martin, G.B., and Stebbins, C.E. (2006). A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. Science *311*, 222–226.

Jentsch, S., and Pyrowolakis, G. (2000). Ubiquitin and its kin: How close are the family ties? Trends Cell Biol. *10*, 335–342.

Kattenhorn, L.M., Korbel, G.A., Kessler, B.M., Spooner, E., and Ploegh, H.L. (2005). A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family Herpesviridae. Mol. Cell *19*, 547–557.

Kerscher, O., Felberbaum, R., and Hochstrasser, M. (2006). Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu. Rev. Cell Dev. Biol. *22*, 159–180.

Kim, D.W., Lenzen, G., Page, A.L., Legrain, P., Sansonetti, P.J., and Parsot, C. (2005). The Shigella flexneri effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. Proc. Natl. Acad. Sci. USA *102*, 14046–14051.

Knodler, L.A., Finlay, B.B., and Steele-Mortimer, O. (2005). The Salmonella effector protein SopB protects epithelial cells from apoptosis by sustained activation of Akt. J. Biol. Chem. *280*, 9058–9064.

Kubori, T., and Galan, J.E. (2003). Temporal regulation of salmonella virulence effector function by proteasome-dependent protein degradation. Cell *115*, 333–342.

Kuhle, V., and Hensel, M. (2004). Cellular microbiology of intracellular Salmonella enterica: Functions of the type III secretion system encoded by Salmonella pathogenicity island 2. Cell. Mol. Life Sci. *61*, 2812–2826.

Lee, L.C., Lee, Y.L., Leu, R.J., and Shaw, J.F. (2006). Functional role of catalytic triad and oxyanion hole-forming residues on enzyme activity of Escherichia coli thioesterase I/protease I/phospholipase L1. Biochem. J. *397*, 69–76.

Mansouri, M., Bartee, E., Gouveia, K., Hovey Nerenberg, B.T., Barrett, J., Thomas, L., Thomas, G., McFadden, G., and Fruh, K. (2003). The PHD/LAP-domain protein M153R of myxomavirus is a ubiquitin ligase that induces the rapid internalization and lysosomal destruction of CD4. J. Virol. *77*, 1427–1440.

Marcus, S.L., Knodler, L.A., and Finlay, B.B. (2002). Salmonella enterica serovar Typhimurium effector SigD/SopB is membrane-associated and ubiquitinated inside host cells. Cell. Microbiol. *4*, 435–446.

Melroe, G.T., DeLuca, N.A., and Knipe, D.M. (2004). Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. J. Virol. *78*, 8411–8420.

Mills, S.D., Boland, A., Sory, M.P., van der Smissen, P., Kerbourch, C., Finlay, B.B., and Cornelis, G.R. (1997). Yersinia enterocolitica induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. Proc. Natl. Acad. Sci. USA *94*, 12638–12643.

Misaghi, S., Balsara, Z.R., Catic, A., Spooner, E., Ploegh, H.L., and Starnbach, M.N. (2006). Chlamydia trachomatis-derived deubiquitinating enzymes in mammalian cells during infection. Mol. Microbiol. *61*, 142–150.

Mittal, R., Peak-Chew, S.Y., and McMahon, H.T. (2006). Acetylation of MEK2 and IkappaB kinase (IKK) activation loop residues by YopJ inhibits signaling. Proc. Natl. Acad. Sci. USA *103*, 18574–18579.

Monack, D.M., Mecsas, J., Ghori, N., and Falkow, S. (1997). Yersinia signals macrophages to undergo apoptosis and YopJ is necessary for this cell death. Proc. Natl. Acad. Sci. USA *94*, 10385–10390.

Mukherjee, S., Keitany, G., Li, Y., Wang, Y., Ball, H.L., Goldsmith, E.J., and Orth, K. (2006). Yersinia YopJ acetylates and inhibits kinase activation by blocking phosphorylation. Science *312*, 1211–1214.

Muller, S., Hoege, C., Pyrowolakis, G., and Jentsch, S. (2001). SUMO, ubiquitin's mysterious cousin. Nat. Rev. Mol. Cell Biol. *2*, 202–210.

Neish, A.S., Gewirtz, A.T., Zeng, H., Young, A.N., Hobert, M.E., Karmali, V., Rao, A.S., and Madara, J.L. (2000). Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science *289*, 1560–1563.

Nijman, S.M., Luna-Vargas, M.P., Velds, A., Brummelkamp, T.R., Dirac, A.M., Sixma, T.K., and Bernards, R. (2005). A genomic and functional inventory of deubiquitinating enzymes. Cell *123*, 773–786.

Ohmura-Hoshino, M., Goto, E., Matsuki, Y., Aoki, M., Mito, M., Uematsu, M., Hotta, H., and Ishido, S. (2006). A novel family of membrane-bound E3 ubiquitin ligases. J. Biochem. (Tokyo) *140*, 147–154.

Okuda, J., Toyotome, T., Kataoka, N., Ohno, M., Abe, H., Shimura, Y., Seyedarabi, A., Pickersgill, R., and Sasakawa, C. (2005). Shigella effector IpaH9.8 binds to a splicing factor U2AF(35) to modulate host immune responses. Biochem. Biophys. Res. Commun. *333*, 531–539.

Orth, K., Palmer, L.E., Bao, Z.Q., Stewart, S., Rudolph, A.E., Bliska, J.B., and Dixon, J.E. (1999). Inhibition of the mitogen-activated protein kinase kinase superfamily by a Yersinia effector. Science *285*, 1920–1923.

Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., Mangel, W.F., Staskawicz, B., and Dixon, J.E. (2000). Disruption of signaling by Yersinia effector YopJ, a ubiquitin-like protein protease. Science *290*, 1594–1597.

Ovaa, H., Galardy, P.J., and Ploegh, H.L. (2005). Mechanism-based proteomics tools based on ubiquitin and ubiquitin-like proteins: Synthesis of active site-directed probes. Methods Enzymol. *399*, 468–478.

Palmer, L.E., Hobbie, S., Galan, J.E., and Bliska, J.B. (1998). YopJ of Yersinia pseudotuberculosis is required for the inhibition of macrophage TNF-alpha production and downregulation of the MAP kinases p38 and JNK. Mol. Microbiol. *27*, 953–965.

Patel, J.C., and Galan, J.E. (2006). Differential activation and function of Rho GTPases during Salmonella-host cell interactions. J. Cell Biol. *175*, 453–463.

Perrin, A.J., Jiang, X., Birmingham, C.L., So, N.S., and Brumell, J.H. (2004). Recognition of bacteria in the cytosol of Mammalian cells by the ubiquitin system. Curr. Biol. *14*, 806–811.

Pickart, C.M. (1997). Targeting of substrates to the 26S proteasome. FASEB J. *11*, 1055–1066.

Rohde, J.R., Breitkreutz, A., Chenal, A., Sansonetti, P.J., and Parsot, C. (2007). Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. Cell Host & Microbe *1*, this issue, 77–83.

Rytkönen, A., Poh, P., Garmendia, J., Boyle, C., Thompson, A., Liu, M., Freemont, P., Hinton, J.C.D., and Holden, D.W. (2007). SseL, a *Salmonella* deubiquitinase required for macrophage killing and virulence. Proc. Natl. Acad. Sci. USA *104*, 3502–3507.

Sapetschnig, A., Rischitor, G., Braun, H., Doll, A., Schergaut, M., Melchior, F., and Suske, G. (2002). Transcription factor Sp3 is silenced through SUMO modification by PIAS1. EMBO J. *21*, 5206–5215.

Schnupf, P., Portnoy, D.A., and Decatur, A.L. (2006). Phosphorylation, ubiquitination and degradation of listeriolysin O in mammalian cells: Role of the PEST-like sequence. Cell. Microbiol. *8*, 353–364.

Seeler, J.S., and Dejean, A. (2001). SUMO: Of branched proteins and nuclear bodies. Oncogene *20*, 7243–7249.

Shen, Y., Naujokas, M., Park, M., and Ireton, K. (2000). InlB-dependent internalization of Listeria is mediated by the Met receptor tyrosine kinase. Cell *103*, 501–510.

Sulea, T., Lindner, H.A., and Menard, R. (2006). Structural aspects of recently discovered viral deubiquitinating activities. Biol. Chem. *387*, 853–862.

Taylor, J.M., and Barry, M. (2006). Near death experiences: Poxvirus regulation of apoptotic death. Virology *344*, 139–150.

Terebiznik, M.R., Vieira, O.V., Marcus, S.L., Slade, A., Yip, C.M., Trimble, W.S., Meyer, T., Finlay, B.B., and Grinstein, S. (2002). Elimination of host cell PtdIns(4,5)P(2) by bacterial SigD promotes membrane fission during invasion by Salmonella. Nat. Cell Biol. *4*, 766–773.

Thrower, J.S., Hoffman, L., Rechsteiner, M., and Pickart, C.M. (2000). Recognition of the polyubiquitin proteolytic signal. EMBO J. *19*, 94–102.

Toyotome, T., Suzuki, T., Kuwae, A., Nonaka, T., Fukuda, H., Imajoh-Ohmi, S., Toyofuku, T., Hori, M., and Sasakawa, C. (2001). Shigella protein IpaH(9.8) is secreted from bacteria within mammalian cells and transported to the nucleus. J. Biol. Chem. *276*, 32071–32079.

Tzfira, T., Vaidya, M., and Citovsky, V. (2004). Involvement of targeted proteolysis in plant genetic transformation by Agrobacterium. Nature *431*, 87–92.

Veiga, E., and Cossart, P. (2005). Listeria hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. Nat. Cell Biol. *7*, 894–900.

Wang, J., Loveland, A.N., Kattenhorn, L.M., Ploegh, H.L., and Gibson, W. (2006). High-molecular-weight protein (pUL48) of human cytomegalovirus is a competent deubiquitinating protease: Mutant viruses altered in its active-site cysteine or histidine are viable. J. Virol. *80*, 6003–6012.

Waterman, S.R., and Holden, D.W. (2003). Functions and effectors of the Salmonella pathogenicity island 2 type III secretion system. Cell. Microbiol. *5*, 501–511.

Wood, M.W., Jones, M.A., Watson, P.R., Siber, A.M., McCormick, B.A., Hedges, S., Rosqvist, R., Wallis, T.S., and Galyov, E.E. (2000). The secreted effector protein of Salmonella dublin, SopA, is translocated into eukaryotic cells and influences the induction of enteritis. Cell. Microbiol. *2*, 293–303.

Yeh, E.T., Gong, L., and Kamitani, T. (2000). Ubiquitin-like proteins: New wines in new bottles. Gene *248*, 1–14.

Yoon, S., Liu, Z., Eyobo, Y., and Orth, K. (2003). Yersinia effector YopJ inhibits yeast MAPK signaling pathways by an evolutionarily conserved mechanism. J. Biol. Chem. *278*, 2131–2135.

Zhang, Y., Higashide, W., Dai, S., Sherman, D.M., and Zhou, D. (2005). Recognition and ubiquitination of Salmonella type III effector SopA by a ubiquitin E3 ligase, HsRMA1. J. Biol. Chem. *280*, 38682–38688.

Zhang, Y., Higashide, W.M., McCormick, B.A., Chen, J., and Zhou, D. (2006). The inflammation-associated Salmonella SopA is a HECT-like E3 ubiquitin ligase. Mol. Microbiol. *62*, 786–793.

Zhou, L., Tan, A., and Hershenson, M.B. (2004). Yersinia YopJ inhibits pro-inflammatory molecule expression in human bronchial epithelial cells. Respir Physiol Neurobiol *140*, 89–97.

Zhou, H., Monack, D.M., Kayagaki, N., Wertz, I., Yin, J., Wolf, B., and Dixit, V.M. (2005). Yersinia virulence factor YopJ acts as a deubiquitinase to inhibit NF-kappa B activation. J. Exp. Med. *202*, 1327–1332.