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Perspective

VCP/p97 Mediated Bacteriolysis: A Ubiquitin-Powered Mechanical Arm of Cell-Autonomous Immunity

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Abstract: Intracellular pathogens that escape into the cytosol challenge classical immune defenses. Host cells counteract this threat through ubiquitin-mediated recognition and elimination. Recent work by Ghosh et al. highlights VCP/p97, a host AAA ATPase, as a cytosolic effector that translates ubiquitin recognition into mechanical disruption of bacterial integrity. In this perspective, we discuss a previously unrecognized strategy for broad-spectrum pathogen control by mechanical force generation, expanding on our current views on ubiquitin-mediated immunity.

Keywords: cell-autonomous immunity; AAA-ATPase; VCP/p97; ubiquitin; proteasome; immune effectors; bacteriolysis

1. Introduction

The innate immune system employs a multilayered strategy to eliminate a variety of foreign threats. These include nutrient deprivation, compartmentalization, and a diverse array of chemical and enzymatic strategies, which form a robust first line of defense. However, successful pathogens have evolved to outwit many of these defenses by inducing endocytosis in non-professional phagocytes followed by escape into the cytosol. This not only allows evasion from extracellular death traps but also promotes possibilities for establishment of a cytosolic niche, facilitating dissemination into deeper tissues and disease progression. This evolutionary pressure has driven the emergence of cytosolic immune effectors that detect and eliminate the threat in a fail-safe manner. A recent study by Ghosh et al. reveals a previously underappreciated antibacterial function of the cytosolic AAA-ATPase VCP/p97 (Valosin-containing protein). While traditionally involved in maintaining cellular homeostasis, this study surprisingly found that VCP/p97 targets ubiquitinated surface proteins of cytosolic bacteria to inflict mechanical damage that irreversibly compromises bacterial integrity (Figure 1) [1].

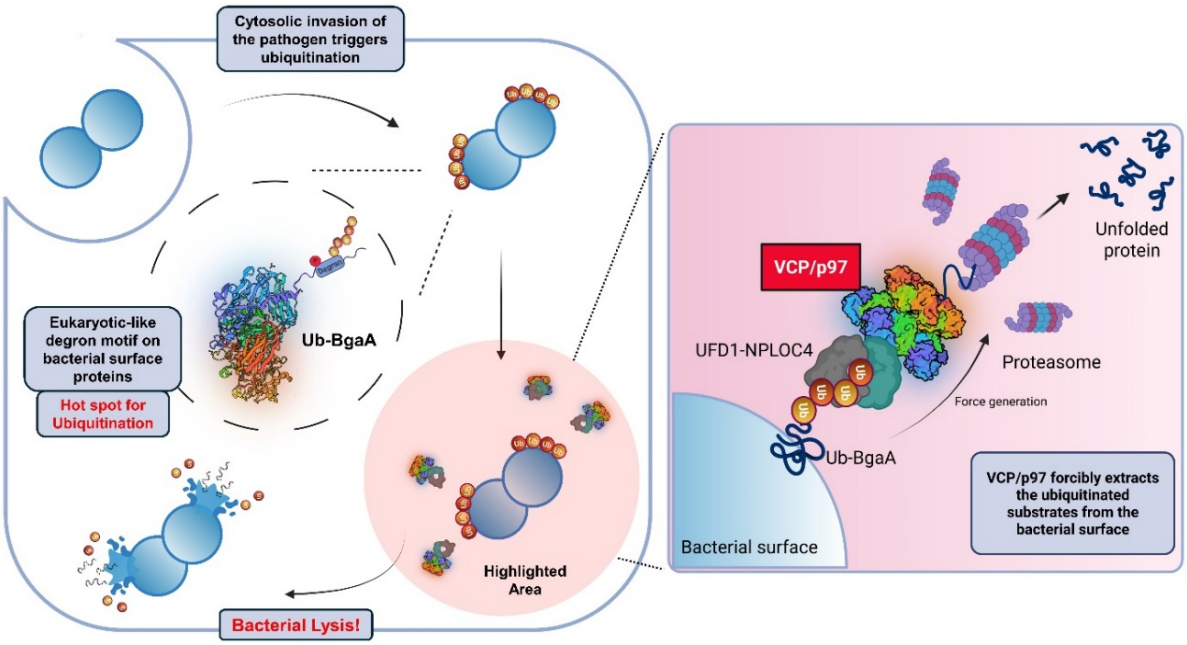

Figure 1. Following endocytosis, a few bacterial pathogens rupture the pathogen-containing vacuolar membrane using pore-forming toxins or needle-like secretion systems to escape into the cytosol. Host ubiquitin ligases, including the SCF^{FBXW7} complex recognize eukaryotic-like degron sequences on the *Streptococcus pneumoniae* surface protein BgaA, thereby conjugating K48 poly-Ub chains to the substrate. Ubiquitination promotes the recruitment of cofactors, such as UFD1 and NPLOC4, which in turn recruit the AAA ATPase VCP/p97 to assemble a tripartite complex on the bacterial surface. Powered by ATP hydrolysis, VCP/p97 converts chemical energy into mechanical force to unfold and extract ubiquitinated surface proteins, triggering destabilization of the bacterial membrane and driving pathogen disintegration.

2. The Multifaceted Nature of Cytosolic Defense

Cytosol invading pathogens pose a significant challenge to the host as they escape from the endosomal compartment via expression of pore-forming toxins or needle-like secretion systems, rendering the lysosomal-mediated degradation, the classical antimicrobial mechanism, ineffective. This enables pathogens to utilize host cytosolic resources and proliferate. However, host cells counter this challenge by deploying several specialized cytosolic surveillance systems, including metabolic remodelling, biomolecular condensate formation, translational reprogramming, membrane repair, and autophagy, to restrict intracellular bacterial proliferation [2]. For example, early membrane damage is marked by aberrant exposure of sphingomyelin on the cytosolic face of the damaged endosome containing bacteria, which is sensed by TECPR1 (Tectonin beta-propeller repeat containing 1) to promote LC3 lipidation [3,4]. More extensive damage exposes luminal glycans that are normally shielded within the vacuole, which are detected by cytosolic galectins, notably galectin-3 and galectin-8, thereby recruiting autophagy receptor NDP52 (Nuclear domain 10 protein 52) to drive xenophagy [5]. In parallel, interferon-inducible GTPases (IRGs) contribute to vacuolar pathogen control. IRGM1, for example, binds phosphoinositide and facilitates the assembly of the GKS family IRGs on pathogen-containing phagosomes, thereby promoting lysosomal fusion and pathogen clearance [6]. When vacuolar damage becomes extensive, the pathogen escapes freely into the cytosol, allowing the host cells to deploy specialized defense effectors. Among these, guanylate-binding proteins (GBPs) have emerged as a central effector capable of targeting the Lipopolysaccharide (LPS) of Gram-negative bacteria. Following IFNγ stimulation, GBP1 inserts into the LPS-rich outer membrane and nucleates the assembly of additional GBPs, including GBP2, GBP3 and GBP4, thereby promoting caspase-4 activation. Activated caspase-4 cleaves Gasdermin D, leading to pyroptotic cell death [7]. Beyond inflammasome activation, it has been implicated that mouse GBPs harboring N-terminal hydrophobic amino acid residues are involved in damaging the surface of *Francisella spp* [8]. Since gram-negative bacteria possess a double membrane, disrupting the outer membrane alone by GBPs is insufficient for pathogen clearance. To overcome this, apolipoprotein L3 (APOL3) synergizes with GBP to breach the inner membrane of *Salmonella typhimurium* and break it down into micellar structures [9]. Moreover, during infection, proteasomes can be functionally rewired by the recruitment of the proteasomal activator subunit (PSME3), thereby enhancing the production of proteasome-derived antimicrobial peptides enriched in cationic residues. These peptides bind to the bacterial membrane,

compromising its integrity and suppressing bacterial proliferation [10]. Despite their potencies, these strategies remain limited to majorly LPS-harboring pathogens. Moreover, several pathogens actively counter GBPs by secreting ubiquitin ligase-like effectors, such as Invasion Plasmid Antigen H9.8 (IpaH9.8) from *Shigella flexneri* and Yersinia Secreted E3 Ligase 1/2 (YspE1/2) from *Yersinia pestis*, which target GBPs for proteasomal degradation [11,12], thereby suppressing inflammation and promoting bacterial survival. This constraint has shifted attention towards ubiquitination as a more universally deployed cytosolic defense system, capable of sensing and eliminating a broad spectrum of bacterial pathogens.

3. Ubiquitination as a Bacterial Sensing Strategy

For over two decades, the ubiquitin system has been recognized as a crucial component of cell-autonomous immunity in restricting the growth of cytosolic bacteria. Ubiquitination is a key post-translational modification in which ubiquitin, an 8.6 kDa protein, is attached to the lysine residue of the substrate as a monomer or polymerized into chains of diverse linkage types, via its C-terminal glycine residue, forming an isopeptide bond through the sequential action of E1, E2 and E3 ligases [13]. During intracellular infection, dedicated host cell enzymes catalyze the conjugation of polyubiquitin chains to cytosol exposed microbes, thereby marking the pathogen for downstream immune responses. For example, the E3 ligase SMURF1 (SMAD Specific E3 Ubiquitin Protein Ligase 1) promotes the K48-polyubiquitination of *Mycobacterium tuberculosis* [14]. Likewise, Parkin decorates bacteria containing vacuoles with K63-polyUb chains [15], whereas ARIH1 (Ariadne RBR E3 Ubiquitin Protein Ligase 1) [16] and LRSAM1 (Leucine-rich repeat and sterile alpha motif-containing protein 1) [17] modify *Salmonella typhimurium* with K48-polyUb and K6 poly-Ub, respectively. Interestingly, the E3 ligase RNF213 (RING Finger Protein 213) carved out a distinct niche by recognizing non-proteinaceous substrates, such as LPS, thereby adding a layer of flexibility to substrate sensing [18]. Moreover, signatures of intense positive selection have raised the possibility that RNF213 may have evolved to recognize a phylogenetically broad range of pathogens, including bacterial and protozoans [19]. However, the properties of this large enzyme that determine its pathogen range remain to be validated. Further, a direct comparison of pathogen disposal efficiencies across major ubiquitin chain topologies demonstrates that the K48-polyUb chains provide more effective host protection by promoting proteasomal degradation, whereas the K63-polyUb chains primarily facilitate bacterial clearance through xenophagy [20]. In this framework, eukaryotic-like degron motifs present on bacterial surface proteins serve as a novel pathogen-associated molecular pattern (PAMP), allowing mobilization of host ubiquitin ligase machinery for pathogen recognition and subsequent proteasomal clearance. Supporting this model, in *Streptococcus pneumoniae*, the host E3 ligase SCF (Skp1-Cul1-FBXW7) recognizes degron motifs within the bacterial surface protein BgaA (β-galactosidase) and PspA (Pneumococcal surface protein A), catalyzing their modification with K48-polyUb chains [21]. This exemplifies a frugal mechanism that repurposes cellular homeostatic machinery for innate defense.

4. The Missing Molecular Link

But these findings exposed a fundamental paradox in ubiquitin-proteasome-mediated antibacterial defense. Proteasomal degradation requires the substrate to be unfolded and threaded through a narrow central pore, a physical constraint incompatible with the size of an intact bacterium. This mismatch in size implies that the degradation of the entire pathogen via the proteasome is irrational and necessitates extraction of ubiquitinated bacterial surface proteins. As the proteasome itself lacks the ability to extract membrane-bound substrates, these observations point to the need for an auxiliary molecular machine capable of coupling ubiquitin recognition to mechanical extraction. The AAA-ATPase VCP/p97 emerged as the missing molecular link.

5. VCP/p97 as Bacteriolytic Effector

The recent findings by Ghosh et al. extend the paradigm of cell-autonomous immunity, showing that VCP/p97 selectively targets cytosolic or cytosol-exposed bacteria whose surface proteins, including BgaA and PspA, are decorated with K48-polyUb chains. The ubiquitin-binding cofactors NPLOC4 and UFD1 form a tripartite complex with p97 that directly engages the pathogen and extracts ubiquitinated surface-anchored proteins, thereby lysing the bacteria. VCP/p97, a type II AAA+ ATPase, participates in a broad range of cellular quality control pathways, including endoplasmic reticulum-associated degradation (ERAD) [22], mitochondria-associated degradation (MAD) [23], Golgi reassembly [24], chromatin-associated degradation [25], ribosome-associated degradation [26], autophagy [27] and lysophagy [28]. Structurally, VCP/p97 assembles into hexamers comprising of two stacked ATPase rings, D1 and D2, an N-terminal domain and an unstructured/disordered C-terminal tail. Typically, VCP/p97 is guided onto its substrates by various cofactors. These cofactors bind to

VCP/p97 either at its N-terminus via Ubiquitin Regulatory X or X-Like domain (UBX/UBXL), VCP Interacting or Binding Motif (VIM/VBM), and Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (SHP) motifs, or at its C-terminus via Peptide:N-glycanase/UBA or UBX-containing proteins or PLAP, Ufd3p, and Lub1p (PUB/PUL) motifs. These cofactors eventually guide VCP/p97 towards its substrate, either ubiquitin-dependent or independent [29]. This well-established unfoldase activity provides a mechanistic framework for repurposing p97 to extract ubiquitinated proteins from the bacterial surface. Consistent with this mechanism, multiple experimental approaches, including transmission electron microscopy combined with p97-specific immunogold labeling, revealed pronounced deformation of the bacterial surface, while computational simulations support this model by showing that removing the surface protein is sufficient to compromise bacterial cell wall integrity. In parallel, optical trapping assay provided real-time evidence for force-driven bacterial protein extraction. Further, reconstitution of the entire reaction (from bacterial ubiquitination to p97-mediated protein extraction) in in-vitro unambiguously established the active antibacterial role of p97. In cells, p97 specifically targeted cytosol-exposed bacteria, whereas pathogens retained within intact endosomes remain inaccessible to p97 surveillance. Depleting key E3 ligases, such as SCF^{FBXW7} and ARIH1, known to ubiquitinate pathogenic bacteria such as *Streptococcus pneumoniae* and *Salmonella typhimurium*, respectively, demonstrates the necessity of ubiquitination as the fundamental signature required for p97-mediated recognition. The impact of p97 mutations arising in the population linked to multisystem proteinopathy [30] was also explored, showing that selective p97 variants, such as R155H or L198W, fail to clear the bacterial load from the intracellular milieu. At the organismal level, the relevance of this mechanism was validated through in-vivo mice model, showcasing the importance of p97 in the restriction of bacterial proliferation during host during sepsis (Figure 1) [1].

6. Conclusions and Perspective

Collectively, these findings establish VCP/p97 as a key cytosolic bacteriolytic effector that, rather than functioning merely as an adaptor for proteasomal delivery, converts ubiquitin-encoded ‘eat-me’ signals into a conduit for mechanical force-driven pathogen elimination. This work necessitates reevaluation of innate immunity beyond biochemical signaling, broadening the conceptual framework of cell-autonomous defense to encompass mechanical destruction and force-generating nanomachines as fundamental principles of host protection. Nevertheless, how this mechanical defensive strategy interfaces with the broader adaptive immune network remains to be defined. The observation of bacterial targeting hints at additional levels of fine-tuning beyond the contribution of cofactors. Dissecting this regulatory modulus will be essential for understanding how p97’s activity is selectively mobilized without perturbing cellular homeostasis. High-resolution structural studies will be crucial for understanding the precise molecular mechanism and the coordinated force generation required to drive architectural changes at the bacterial surface.

Author Contributions

S.G. and A.B. conceptualized the review, analyzed the literature, wrote, edited, and proofread the manuscript. Both authors approve the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

Acronyms

AAA-ATPase—ATPase Associated with diverse cellular Activities
VCP/p97—Valosin containing protein
Ub—Ubiquitin
UFD1—Ubiquitin fusion degradation protein 1
NPLOC4—Nuclear protein localization protein 4
GBP—Guanylate binding protein
LPS—Lipopolysaccharide
IRGM1—Immunity-related GTPase family M member 1
APOL3—Apolipoprotein L3
ARIH1—Ariadne RBR E3 Ubiquitin Protein Ligase 1
Skp1—S-phase kinase-associated protein 1
Cul1—Cullin 1
FBXW7—F-box and WD repeat domain-containing 7
BgaA—Beta-galactosidase
PspA—Pneumococcal surface protein A

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