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Regulation of Host-Pathogen Interactions via the Ubiquitin System

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Abstract

Ubiquitination is a posttranslational modification that regulates a multitude of cellular functions. Pathogens, such as bacteria and viruses, have evolved sophisticated mechanisms that evade or counteract ubiquitin-dependent host responses, or even exploit the ubiquitin system to their own advantage. This is largely done by numerous pathogen virulence factors that encode E3 ligases and deubiquitinases, which are often used as weapons in pathogen–host cell interactions. Moreover, upon pathogen attack, host cellular signaling networks undergo major ubiquitin-dependent changes to protect the host cell, including coordination of innate immunity, remodeling of cellular organelles, reorganization of the cytoskeleton, and reprogramming of metabolic pathways to restrict growth of the pathogen. Here we provide mechanistic insights into ubiquitin regulation of host-pathogen interactions and how it affects bacterial and viral pathogenesis and the organization and response of the host cell.

Contents

1. INTRODUCTION

1.1. Ubiquitin-Mediated Regulation of Cellular Processes

Ubiquitination is a reversible posttranslational modification that regulates almost all intracellular signaling networks. It is catalyzed by a multienzyme cascade that includes E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) enzymes. In the first step, ubiquitin is activated by formation of a thioester linkage between the carboxy-terminal glycine of ubiquitin and a cysteine residue on the E1 enzyme in an ATP-dependent reaction. This is followed by the transfer of the activated ubiquitin molecule to the E2 enzyme; the E3 ligase then mediates the transfer of ubiquitin to lysine residues of substrates (31). In the cell, there are several

hundred E3 ligases that confer substrate specificity to the ubiquitination process (4, 75, 102, 105). They can be broadly grouped into RING finger–containing ligases, HECT-type ligases, and RING-between-RING (RBR) ligases. Ubiquitin can be conjugated to one or more lysine residues of the substrate (known as monoubiquitination or multi-monoubiquitination). Ubiquitination can also result in polyubiquitin chains created by linking any of the seven lysine residues of ubiquitin (Lys-6, Lys-11, Lys-27, Lys-29, Lys-33, Lys-48, and Lys-63) or the amino-terminal methionine (Met-1) of ubiquitin to the carboxy-terminal glycine residue of another ubiquitin molecule (70). Ubiquitination regulates protein levels by targeting substrates for proteasomal or lysosomal degradation (73). It can also regulate protein function and localization and interactions between proteins. Deubiquitinases antagonize the effect of ubiquitination by hydrolyzing the isopeptide bond between two ubiquitin molecules or the link between ubiquitin and the substrate protein (12).

1.2. Pathogen-Induced Immune Response and Its Ubiquitin-Dependent Modulation

Ubiquitin is an important regulatory factor that governs the interplay between the host defense system and the effector proteins of bacteria and viruses that have evolved to evade host surveillance. Pathogenic invasion remodels ubiquitin-dependent signaling pathways in the host cell. Upon pathogen entry, pattern-recognition receptors (PRRs), such as Toll-like receptors, nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid–inducible gene I (RIG-I)-like receptors (RLRs), sense membrane components or nucleic acids of pathogens and initiate local and systemic inflammatory responses (1, 74). Cytoplasmic DNA can activate cGAS (cyclic GMP-AMP synthase) and its downstream signaling effector STING (stimulator of interferon genes). Activated PRRs initiate signaling via signaling hubs like TBK1 (TANK-binding kinase 1), NF-κB (nuclear factor–κB), and MAPK (mitogen-activated protein kinase). Viral RNA is sensed by RIG-I and MDA5 (melanoma differentiation–associated protein 5). This activates the MAVS (mitochondrial antiviral signaling) protein, which forms clusters on the mitochondrion-associated membranes to initiate signaling via IRF3 (interferon regulatory factor 3), leading to the transcription of type I interferons such as IFN-α/β. These can then activate JAK1/STAT1 signaling, causing the transcriptional upregulation of ISGs (interferon stimulated genes) (**Figure 1**). These innate immunity signaling networks are modulated by the cellular ubiquitination machinery and are the prime targets of bacterial and viral effector proteins that counterattack the host immune response. Such virulence factors include, for example, bacterial and viral E3 ligases and deubiquitinases. Ubiquitin-dependent host-pathogen interactions concerning the innate immune response are well researched and have been reviewed extensively (55, 77) (**Figure 1**).

Importantly, bacterial or viral infection, in addition to activating the innate immune response, leads to significant changes in the morphology, positioning, dynamics, and function of subcellular organelles, the cytoskeleton, and the metabolic pathways of the cell. Many of these events are regulated by host E3 ligases and deubiquitinating enzymes (DUBs) that attempt to defend the cell against the invading pathogen. This review highlights how ubiquitin is used to remodel organelles and reprogram the metabolism of infected cells to either facilitate (pathogen-induced) or hinder (host-induced) proliferation of the pathogen.

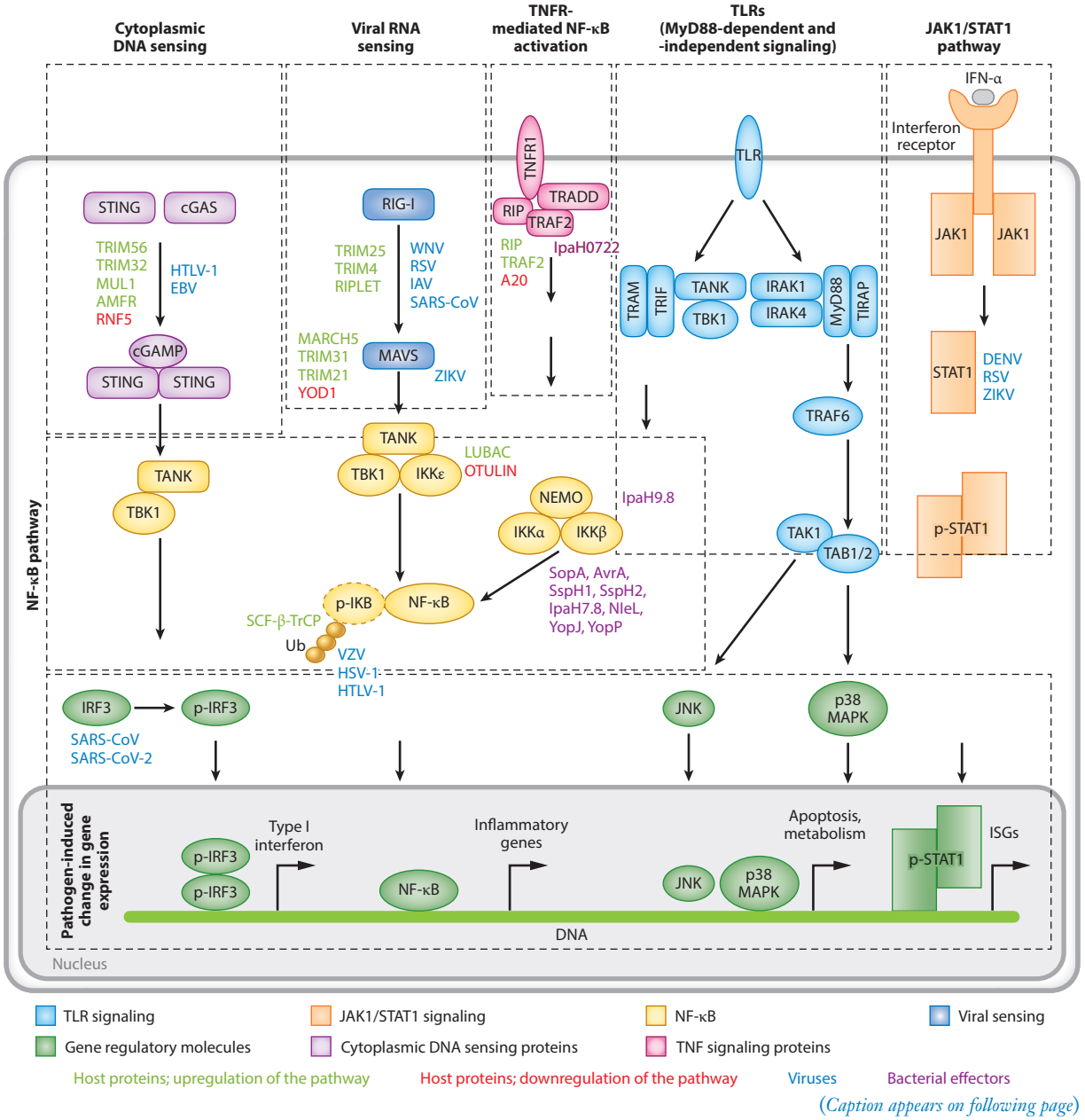
2. UBIQUITIN-MEDIATED REGULATION OF MITOCHONDRIAL MORPHOLOGY AND FUNCTION IN INFECTED CELLS

2.1. Pathogen-Mediated Regulation of Mitochondrial Dynamics

Mitochondria are essential cellular organelles that form a dynamic tubular network that undergoes constant cycles of fission and fusion (65). Mitochondrial fission is mediated by dynamin-related

proteins (DRPs) and cofactors that assemble DRP rings, while fusion is coordinated by the large GTPases mitofusin 1 and 2 (Mfn1 and Mfn2).

Infection of cells with pathogenic bacteria perturbs mitochondrial dynamics, usually causing fragmentation of the mitochondrial network. This fragmentation may be a host response to defend against bacterial infection. Alternatively, mitochondrial fragmentation may be actively induced by bacterial effectors in order to reprogram cellular metabolism and redirect energy resources toward bacterial proliferation. For example, *Legionella pneumophila* secretes an effector called MitF,



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Regulation of innate immunity pathways by cellular E3 ligases, deubiquitinating enzymes, and pathogenic proteins that affect ubiquitin-dependent regulation. Upon infection, the cytoplasmic DNA sensor STING/cGAS, or pattern recognition receptors like RIG-I, and TLRs sense nucleic acids of pathogens. These then activate the NF-κB pathway and the IRF3 pathway, which switch on a transcriptional program that produces an inflammatory and a type I interferon response, respectively. NF-κB-mediated signaling can also be activated by TNF-α binding to TNFR. IFN-α/β produced downstream of IRF3 signaling can activate JAK/STAT signaling, which causes transcriptional upregulation of antiviral genes called ISGs. All these pathways are regulated by host proteins (*green*, upregulation; *red*, downregulation) and pathogens/pathogenic effectors (*purple*, bacterial effectors; *blue*, viruses). Abbreviations: β-TrCP, β-transducin repeat–containing protein; cGAMP, cyclic-GMP-AMP; cGAS, cyclic GMP-AMP synthase; DENV, dengue virus; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus 1; HTLV-1, human T-lymphotropic virus 1; IAV, influenza A virus; IFN-α, interferon-α; IRF3, interferon regulatory factor 3; ISG, interferon-stimulated gene; LUBAC, linear ubiquitin assembly complex; MAPK, mitogen-activated kinase; MARCH5, membrane-associated RING-CH 5; MAVS, mitochondrial antiviral signaling; NF-κB, nuclear factor–κB; RIG-I, retinoic acid–inducible gene I; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SCF, SKP1-CUL1-F-box; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TRIM, tripartite motif–containing; Ub, ubiquitin; VZV, varicella zoster virus; WNV, West Nile virus; ZIKV, Zika virus.

which causes DRP1-dependent fission of mitochondria (22). In this case the increase in mitochondrial fission does not lead to loss of mitochondrial membrane potential ($\Delta\psi_m$). This is because *Legionella* alters the function of the mitochondrial F1F0 ATPase to make it function in reverse mode. Therefore, instead of synthesizing ATP, the F1F0 synthase hydrolyzes ATP to maintain $\Delta\psi_m$. This delays cell death and ensures bacterial proliferation (21). Some bacteria, such as *Chlamydia trachomatis*, induce mitochondrial hyperfusion through miR-30c-5p–dependent inhibition of Drp1, which increases the availability of mitochondrion-derived ATP to *Chlamydia* (11) (**Figure 2*a*,*c***).

2.2. Bacterial Effectors That Modulate Mitochondrial Quality Control

Malfunctioning mitochondria are recognized by a microtubule-associated protein light chain 3 (LC3), in a ubiquitin-dependent or -independent manner (**Figure 1**). In turn, mitophagy receptors, which harbor an LC3-interacting region (LIR) motif, associate directly with LC3 and promote autophagosome formation (28).

Several E3 ligases [MARCH5 (membrane-associated RING-CH 5), PARKIN, SIAH2, MUL1, AMFR, and RNF185] and deubiquitinases (USP30, USP15, USP8, and OTUD6A) regulate mitochondrial function and quality control via mitophagy (28). Upon pathogen attack, this ubiquitin-related quality control machinery is often redirected toward surveillance against invading pathogens. For example, the E3 ligase PARKIN, which is important in mitophagy, can also ubiquitinate intracellular bacteria during *Mycobacterium tuberculosis* infection to target them for xenophagy, i.e., selective autophagy (57). Accordingly, PARKIN-deficient mice are more susceptible to *Streptococcus pneumoniae* infection, exhibiting higher bacterial burden and severe lung damage 2 days after infection (108). In *Listeria*-infected cells, the pore-forming toxin listeriolysin O (LLO) interacts with an NLR on mitochondria called NLRX1 to induce mitophagy. In this case, induction of mitophagy promotes intracellular survival of the pathogen (59). Antiviral signaling also utilizes E3 ligases involved in mitophagy: MUL1 and AMFR can cause ubiquitination of STING to activate TBK1-mediated interferon response in virus-infected cells (100).

2.3. Mitochondrial ROS and Activation of the Inflammasome

Bacteria like *Helicobacter pylori* and *Listeria monocytogenes* secrete pore-forming toxins, such as VacA and LLO, which lead to mitochondrial outer membrane permeabilization and release of cytochrome *c* (26, 89). This is accompanied by release of mitochondrial reactive oxygen species (ROS) and activation of the inflammasome (**Figure 2*c***). The NLRP3 inflammasome is an

intracellular PRR that is activated by dysfunctional mitochondria. NLRP3 senses mitochondrial ROS and localizes to damage sites, where it promotes release of mitochondrial DNA (mtDNA) into the cytosol. The activated inflammasome then initiates to an inflammatory response via caspase-1 activation, resulting in the secretion of proinflammatory cytokines like interleukin-1β (IL-1β) and IL-18 (109). Bacterial infection also activates the host Toll-like receptor signaling, causing ubiquitination of the mitochondrial respiratory chain assembly factor ECSIT, which leads to complex I–mediated ROS generation. ROS are bactericidal and reduce the bacterial load in *Salmonella enterica* Typhimurium–infected cells (10, 103). Moreover, mitochondrial ROS

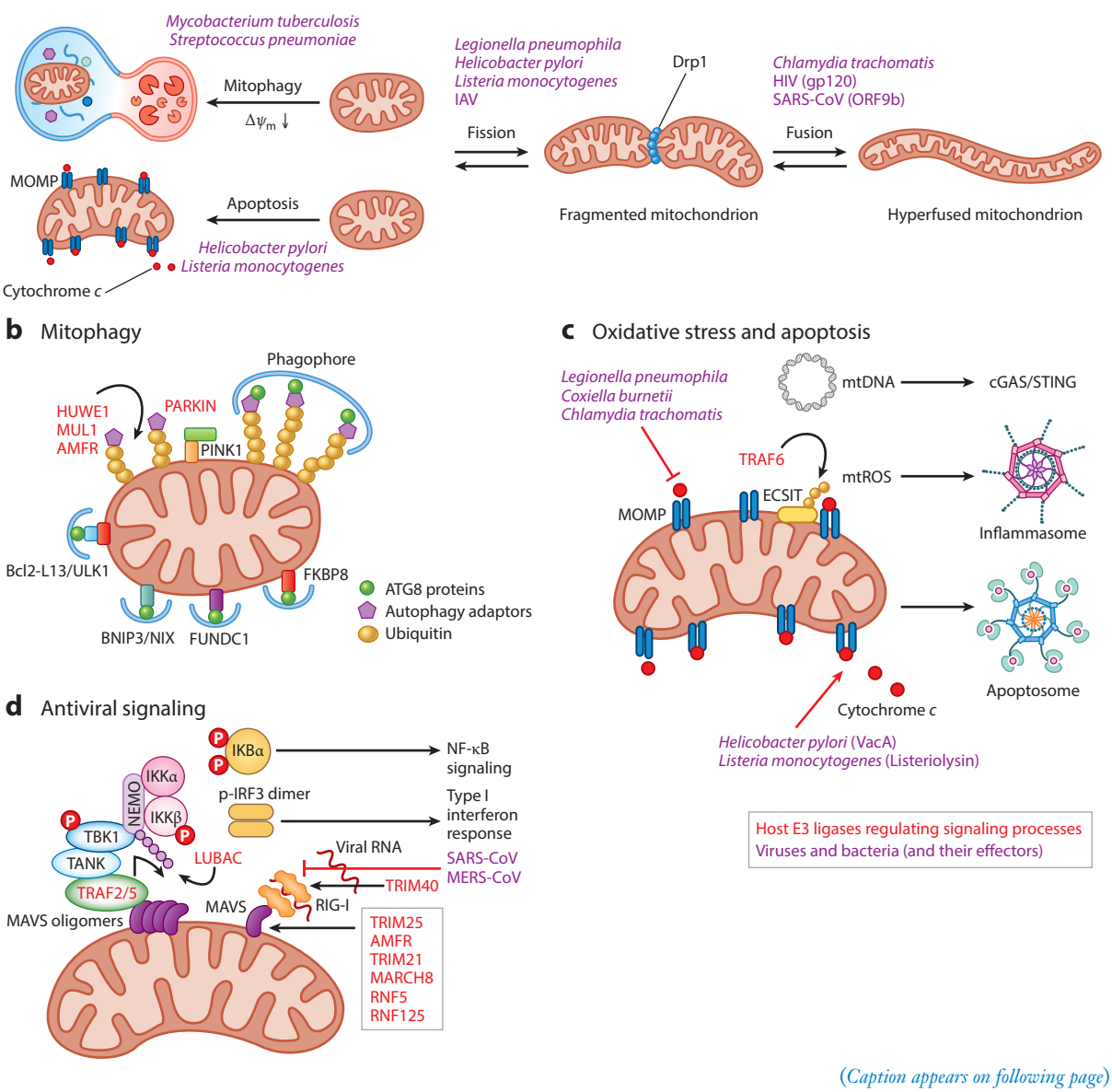


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Figure 2 (*Figure appears on preceding page*)

Mitochondrial signaling in bacterial and viral infection. (*a*) Pathogens modulate the host mitochondria by tilting the fission-fusion balance toward increased fission (more common) or increased fusion (as caused by *Chlamydia trachomatis* and some viruses). (*b*) Regulation of mitophagy by cellular E3 ligases and DUBs; this machinery is often redirected toward pathogen-specific autophagy (xenophagy). (*c*) Pathogens and host cells regulate mitochondrial ROS production, mitochondrial outer membrane permeabilization, and mtDNA release, which in turn can activate the inflammasome or the intrinsic pathway of apoptosis. (*d*) MAVS present on the mitochondria acts as antiviral signaling platforms and is regulated by several cellular E3 ligases and DUBs. Viruses like SARS-CoV and MERS-CoV have evolved ways to evade activation of MAVS signaling. Virus and bacteria (and their effectors) are written in purple, host E3 ligases regulating signaling processes are in red. Abbreviations: cGAS, cyclic GMP-AMP synthase; DUB, deubiquitinating enzyme; IAV, influenza A virus; IRF3, interferon regulatory factor 3; LUBAC, linear ubiquitin assembly complex; MARCH8, membrane-associated RING-CH 8; MAVS, mitochondrial antiviral signaling; MERS-CoV, Middle East respiratory syndrome coronavirus; MOMP, mitochondrial outer membrane permeabilization; mtROS, mitochondrial ROS; NF-κB, nuclear factor–κB; RIG-I, retinoic acid–inducible gene I; ROS, reactive oxygen species; SARS-CoV, severe acute respiratory syndrome coronavirus; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TRIM, tripartite motif–containing.

lead to deubiquitination and subsequent activation of NLRP3. Deubiquitination is catalyzed by ABRO1, a part of the BRISC ubiquitinase complex. ABRO1 binding to NLRP3 in an Ser-194 phosphorylation–dependent manner recruits BRISC to remove Lys-63-linked ubiquitin chains of NLRP3 in response to LPS stimulation (41, 76). mtDNA released upon mitochondrial fragmentation has been shown to activate the NLRC4 inflammasome in *Pseudomonas aeruginosa*–infected cells (37). *Coxiella burnetii* blocks mitochondrial outer membrane permeabilization by utilizing type IV secretion system (T4SS) effectors CaeA, CaeB, and AnkG. AnkG binds the host mitochondrial protein p32 and is translocated to the nucleus upon apoptosis induction, which is believed to exert an antiapoptotic effect (19). The molecular basis of CaeA- and CaeB-mediated inhibition of apoptosis remains unclear; it occurs downstream of Bax activation and blocks the activation of caspases (44). The *Shigella* E3 ligase IpaH7.8 activates caspase-1-mediated cell death by ubiquitinating the inflammasome inhibitor glomulin (93).

2.4. Mitochondrial Antiviral Signaling

Mitochondria are an important platform for antiviral signaling (**Figure 2*d***). The mitochondrial antiviral signaling (MAVS) protein forms prion-like aggregates that are crucial for eliciting a type I interferon response in virus-infected cells. MAVS-dependent antiviral signaling is initiated after the RLRs RIG-I and MDA5 sense viral RNA in the cell. MAVS signaling is regulated by several posttranslational modifications, including ubiquitination. RIG-I activation requires the E3 ubiquitin ligases Riplet and TRIM25 (tripartite motif–containing protein 25), which ubiquitinate RIG-I by ubiquitin linkages. MAVS is known to be ubiquitinated by several E3 ligases: TRIM25, ITCH, MARCH5, RNF5, and RNF125. TRIM25-mediated ubiquitination is necessary for MAVS-dependent signaling, whereas the other ligases cause proteasomal degradation of MAVS. This makes TRIM25, in addition to MAVS, a target of viral effector proteins. The N protein of SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (Middle East respiratory syndrome coronavirus) interacts with the C-terminal SPRY region of TRIM25 and blocks the activation of RIG-I. Moreover, Orf9b of SARS-CoV causes ubiquitination and disassembly of the MAVS signalosome by usurping the cellular E3 ligase ITCH (84). The hepatitis C virus (HCV) NS3-NS4A protease complex inactivates MAVS signaling by cleaving MAVS at Cys-508, preventing its targeting to the membrane.

MARCH ligases, including MARCH5, are also vital players in antiviral immunity. MARCH5 is present on the outer mitochondrial membrane and regulates Drp1-dependent mitochondrial fission and endoplasmic reticulum (ER)-mitochondrion contacts. In vesicular stomatitis virus (VSV)-infected cells, MARCH5 attenuates the interferon response through ubiquitination and degradation of MAVS aggregates (107). MARCH5 also targets aggregates of the viral protein

HBx in cells infected with hepatitis B virus. This ameliorates HBx-mediated ROS production and inflammatory signaling (106).

Finally, the ligase RNF185 has an important role in sensing viral DNA of HSV-1 (herpes simplex virus 1) through the cGAS-STING pathway. RNF185 polyubiquitinates cGAS with Lys-27 ubiquitin chains; this increases cGAS-dependent activation of STING and leads to downstream interferon signaling (99).

3. UBIQUITIN-MEDIATED REGULATION OF THE ENDOPLASMIC RETICULUM AND GOLGI APPARATUS DURING INFECTION

3.1. Ubiquitin-Mediated Regulation of ER Structure

The ER is a vast network of sheets, tubules, and matrices that are remodeled by bacteria to subvert membranes needed for their intracellular survival. Several intracellular bacteria, including *L. pneumophila*, *Legionella longbeachae*, *Brucella* spp., *C. trachomatis*, and the *Chlamydia*-related bacterium *Simkania negevensis*, survive and replicate within ribosome-studded intracellular vacuoles, which are derived from and closely interact with the ER (81). *L. pneumophila* has several effector proteins that intercept ubiquitin signaling in the host cell, resulting in rerouting of ER proteins toward the bacterial vacuole. SidC is an E3 ligase of *L. pneumophila* that is present on the cytosolic side of *Legionella*-containing vacuoles (LCVs) and is important for recruiting host ER proteins and polyubiquitin conjugates to the LCV (32). The bacteria also secrete two deubiquitinases, LotA and LotB, belonging to the ovarian tumor (OTU) superfamily of cysteine proteases, which regulate ubiquitin signatures on the surface of LCVs during infection. LotB deubiquitinates the host SNARE protein SEC22B, which alters interactions between the bacterial vacuole and the ER-derived vesicles (43, 71, 85). Apart from conventional ligases, *Legionella* has a unique family of SidE ligases that can catalyze a noncanonical form of ubiquitination called phosphoribose-linked serine ubiquitination. Here, host proteins are ubiquitinated by the addition of phosphoribosylated ubiquitin on serine residues in a NAD-dependent process. Known substrates of phosphoribosyl ubiquitination (PR-Ub) include ER proteins Rab33b, reticulon 3, and FAM134C, the modification of which leads to ER fragmentation and the recruitment of ER proteins to LCVs (8, 47, 71, 86). Deubiquitinases specific to PR-Ub are also encoded by the *Legionella* genome. These include DupA and DupB, which can reverse PR-Ub of host proteins to fine-tune serine ubiquitination levels during the course of infection (86).

3.2. Ubiquitin-Mediated Regulation of ER Function

The ER also has a quality control pathway called ER-associated protein degradation (ERAD), a process that involves retrotranslocation of misfolded or unassembled proteins from the ER into the cytosol for proteasomal degradation. Several bacterial toxins, notably cholera toxin, pertussis toxin, Shiga and Shiga-like toxins, *Pseudomonas* exotoxin, cytolethal distending toxin, and the plant toxin ricin, hijack the ERAD machinery to enter the cytosol of cells. Cholera toxin enters the cell by retrograde trafficking through the trans-Golgi network to the ER. In the ER, it interacts with the components of the ERAD machinery: Derlin-1, HRD1, and AMFR (6, 7). The retrotranslocation complex comprising Derlin-1 and HRD1 unfolds and retrotranslocates the A1 chain of the toxin to the cytosol. Unlike normal retrotranslocation substrates, the A1 chain escapes degradation by the proteasome and rapidly refolds in the cytosol to act as an ADP-ribosyltransferase (34).

Viruses exploit the ER for most phases of their life cycle, including entry, replication, and assembly following productive infection. Polyomaviruses are nonenveloped viruses that utilize the ER protein disulfide isomerase (PDI) to initiate disassembly of the virus in the ER lumen. Many

RNA and DNA viruses belonging to both the enveloped and nonenveloped virus families rearrange the ER membrane to generate a vast array of ER-derived structures—membranous webs with convoluted membranes that act as scaffolds to recruit viral and host proteins needed for viral replication. Each structure is postulated to facilitate viral replication and assembly. Host E3 ligases associated with ERAD are the most prominent viral targets. Human cytomegalovirus utilizes the ligase TMEM129 to ubiquitinate and degrade MHC-I molecules in infected cells (97). The mouse gammaherpesvirus 68 (MHV68) encodes its own E3 ligase called MK3, which is inserted into the host cell ER and causes degradation of MHC-I (30). The HIV-1 protein Vpu recruits β-TrCP (β-transducin repeat–containing protein), the substrate adaptor for the canonical cullin RING SCF-β-TrCP ligase to cause degradation of the HIV-1 cell surface receptor CD4. This leads to a loss of $CD4^+$ T cells and an imbalance in $CD4^+$ T cell homeostasis, which impairs adaptive immune response to viral infection. Coronaviruses, such as mouse hepatitis virus and SARS-CoV-2, use ER membranes to build double-membrane vesicles and convoluted membranes, which are needed for production and release of viral progeny. To usurp ER membranes, they utilize a process called ERAD tuning. In this process, the luminal ERAD regulators EDEM1 and OS-9 bind to SEL1L and are segregated from the ER into LC3-I-positive ER subregions or vesicles called ERAD-tuning vesicles or EDEMosomes, which are used for viral replication (64).

3.3. Ubiquitin-Mediated Subversion of the Secretory Pathway in Infected Cells

The Golgi apparatus undergoes significant changes in infected cells. Often, to counteract the pathogen, the Golgi apparatus becomes dispersed, which initiates activation of the NLRP3 inflammasome. The Golgi apparatus also acts as a signaling platform for activation of STING in response to cytosolic DNA or DNA viruses, which is needed to initiate a type I interferon response. Some pathogens utilize ubiquitin ligases and DUBs that affect the organization and function of the Golgi apparatus in infected cells. These include the *Legionella* effector protein GobX, an E3 ligase with similarity to mammalian U-box proteins. This effector utilizes the host *S*-palmitoylation machinery to localize to the Golgi apparatus (52). The SidE ligases of *Legionella* also have a C-terminal domain that interacts with Golgi membranes, where they modify the Golgi-stacking proteins GRASP55 and GRASP65 by serine ubiquitination. This leads to fragmentation of the Golgi apparatus, which in turn compromises the secretory pathway of the cell (54). The chlamydial deubiquitinase Cdu1 intercepts vesicular trafficking and recruits Golgi membranes to membrane-bound vacuoles called inclusions, in which *Chlamydia* replicates (3).

Viruses that assemble and mature in the ER use the secretory pathway to reach the plasma membrane be released into the extracellular environment. COP-II-dependent transport of virions from the ER to the cell surface via the Golgi apparatus has been noted in egress of mature HCV, rotavirus, and parvovirus particles.

The E3 ligase MARCH8 has broad antiviral activity against HIV-1 and Ebola virus. It blocks the insertion of glycoproteins into newly formed virions by prohibiting complex glycosylation and proteolytic cleavage of these proteins in the Golgi apparatus (95). It also ubiquitinates the influenza A virus (IAV) protein M2, targeting it for lysosomal degradation (53). MARCH8 can also have proviral effects; for example, in the case of HCV, dengue virus, and Zika viruses, MARCH8 ubiquitinates viral proteins and promotes viral replication. Several viruses, including HCV and dengue virus, utilize the classical secretory pathway for egress, while other viruses, such as rotaviruses, can use nonconventional pathways that bypass the Golgi apparatus. HSV encodes its own ubiquitin ligase (ICP0) and a DUB (UL36), along with a viral protein called HSV-2 tegument protein (UL56), which regulates the activity of the NEDD4 E3 ligase, facilitating the release of virions through the secretory pathway (96).

4. UBIQUITINATION-MEDIATED REGULATION OF VESICULAR TRAFFICKING PATHWAYS

4.1. Intracellular Pathogens Hijack Vesicular Trafficking to Derive Membranes to Form Pathogen-Containing Vacuoles

Several bacteria, such as *L. pneumophila* and *Brucella abortus*, usurp vesicular trafficking at the ER-Golgi interface to form *Legionella*-containing vacuoles (LCVs) and *Brucella*-containing vacuoles that resemble the ER. *C. trachomatis* replicates in large vesicles, called inclusions, that are derived from the trans-Golgi network. *S.* Typhimurium–containing vacuoles (SCVs) hijack endosomal trafficking and make extensive contacts with lysosomes (**Figure 3**). SCVs acquire early endosomal markers (Rab5, EEA1, transferrin receptor) 30 min after infection, followed by the acquisition of late endosomal proteins (Rab7, LAMP1, and the vacuolar ATPase complex), but they do not acquire cathepsins. Bacteria such as *L. pneumophila*, *M. tuberculosis*, and *C. trachomatis* have evolved ways to prevent acidification of the phagosome, while others such as *Coxiella burnetii* and *B. abortus* have learned to replicate in acidic compartments.

4.2. Rab GTPases Are Modulated by Ubiquitination

Rab GTPases are common markers of the bacterial phagosome at early stages of infection. In the host cell, they regulate vesicle budding, transport, tethering, and fusion of phagosomes as they move through the endolysosomal pathway. Ubiquitination of Rab proteins (Rab7, Rab5A, and Rab11) has been shown to be important for their functions (48, 87). *S. enterica*, *C. trachomatis*, and *L. pneumophila* possess several effectors that regulate Rab proteins to hijack vesicular trafficking in the host cell. *Legionella* has several E3 ligases and DUBs that regulate Rab proteins by both conventional and phosphoribose-linked serine ubiquitination. SidE ligases of *L. pneumophila* modify several Rab proteins (Rab33b, Rab1, Rab30) by serine ubiquitination. In this case, serine ubiquitination blocks their GTPase activity but does not cause their degradation. This modification can be reversed by DupA/B. Conventional lysine-linked ubiquitination of Rab10 is modulated by the *Legionella* DUB Lem27, which acts in concert with the bacterial E3 ligases SidC and SdcA (8, 43).

4.3. Antigen Presentation Is Regulated by E3 Ligases and by Pathogens

Antigen presentation via MHC-II molecules in antigen-presenting cells also requires trafficking of inactive MHC-II and peptide-bound MHC-II through the endocytic pathway (**Figure 3**). The E3 ligase MARCH1 is an important regulator of MHC-II-mediated antigen presentation. MARCH1 ubiquitinates the C-terminal tail of MHC-II molecules, enabling internalization of MHC-II into intraluminal vesicles and its endolysosomal degradation (78). Surface expression of loaded mature MHC-II and immature empty MHC-II molecules is also regulated by some pathogens. Recent reports show that the *Salmonella* effector SteD downregulates surface expression of MHC-II by interacting with the host E3 ligase TMEM127, which ubiquitinates MHC-II molecules, leading to their proteasomal degradation (2). Viral proteins that downregulate MHC-II-mediated antigen presentation include Nef (HIV-1), US2 (human cytomegalovirus), and A35 (vaccinia virus) (42).

4.4. The ESCRT Pathway Is Regulated by Pathogens

The endosomal sorting complex required for transport (ESCRT) proteins are also important players in vesicular sorting pathways. They are needed for vesicle-budding processes in cytokinesis, autophagy, multivesicular body and extracellular vesicle biogenesis, and nuclear and endolysosomal membrane repair. The ESCRT protein complexes include ESCRT-0, -I, -II, and -III, which work together to interact with ubiquitinated cargo, followed by vesicle budding and scission.

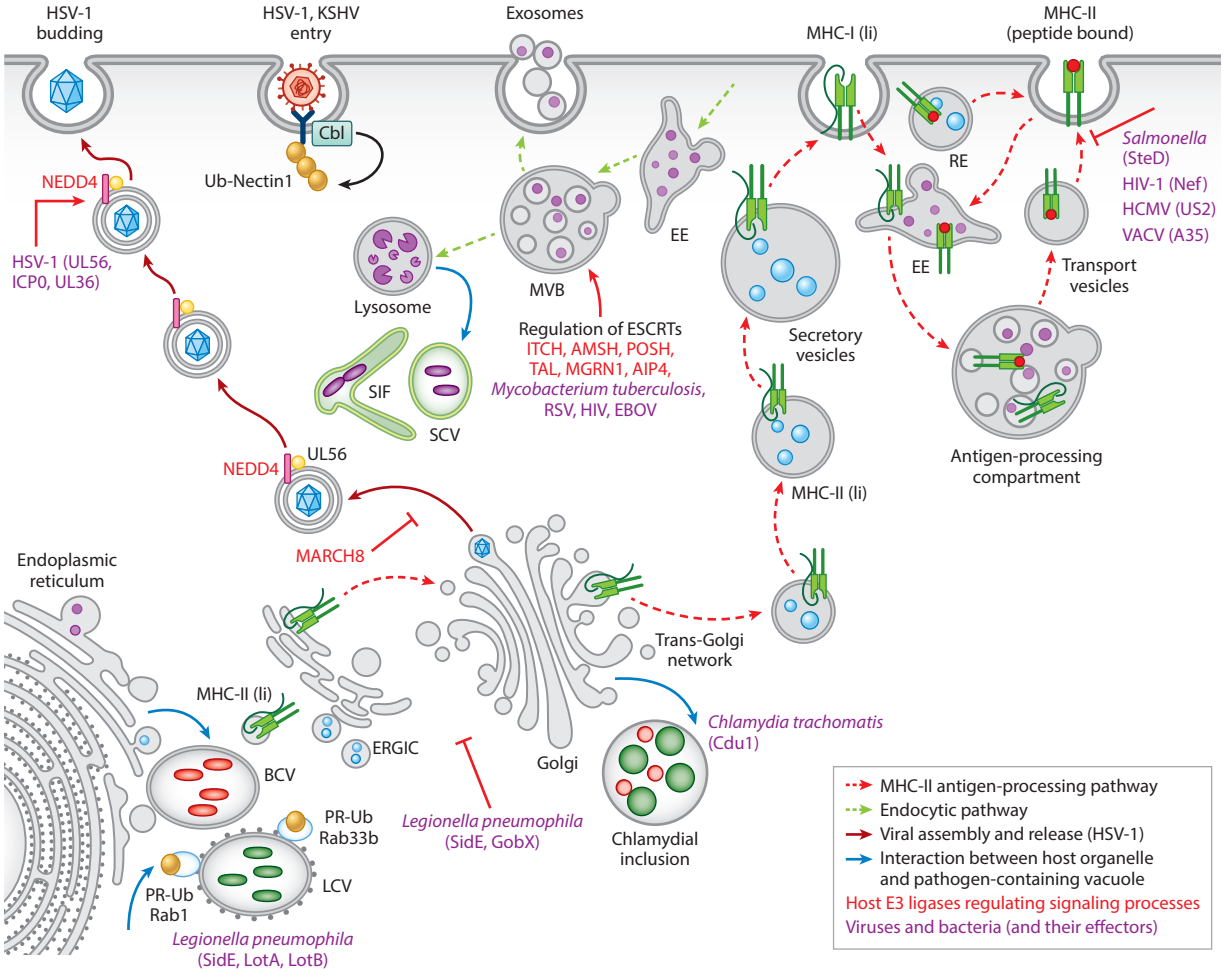

Figure 3

Interaction of pathogens with the vesicular trafficking and the secretory pathway of the host cell. Pathogens hijack different components of the secretory pathway and vesicular trafficking to build vacuoles that serve as a replicative niche. Viruses utilize the secretory pathway for their assembly, maturation, and release. They can also hijack the MHC-II antigen-processing pathway to dampen the adaptive immune response of the host. Host E3 ligases are written in red; pathogens and pathogenic effectors are written in purple. Abbreviations: BCV, *Brucella*-containing vacuole; EBOV, Ebola virus; EE, early endosome; ERGIC, endoplasmic reticulum–Golgi intermediate compartment; HCMV, human cytomegalovirus; HSV-1, herpes simplex virus; KSHV, Kaposi's sarcoma–associated herpesvirus; LCV, *Legionella*-containing vacuole; MARCH8, membrane-associated RING-CH 8; MHC-II (li), MHC-II with invariant chain (inactive); MVB, multivesicular body; PR, phosphoribosyl; RE, recycling endosome; RSV, respiratory syncytial virus; SCV, *Salmonella*-containing vacuole; SIF, *Salmonella*-induced filament; Ub, ubiquitin; VACV, vaccinia virus.

ESCRT-0, -I, and -II proteins have ubiquitin-binding domains that are crucial for their function. ESCRTs also associate with ubiquitin ligases, including NEDD4-family HECT-type ligases, such as AIP4, RSP5, NEDD4, and with the RING ligases MARCH1, POSH, TAL, and Mahogunin (27). The yeast DUB Doa4/Ubp4 deubiquitinates cargo proteins, which regulates the formation of multivesicular bodies (40). The ESCRT machinery is often regulated by pathogens during infection. ESCRT-I components Tsg101 and Vps28 are necessary to restrict intracellular proliferation of bacteria in *Mycobacterium smegmatis*–infected RAW264.7 macrophages (68). Viruses also

regulate ESCRT proteins for viral egress. Several families of enveloped viruses, such as HIV viruses, Ebola viruses, and Rous sarcoma viruses, contain the late assembly domain (L domain) in their Gag proteins, which recruits ESCRT proteins at the site of viral budding. These domains also have a PPXY motif that binds to a subset of NEDD4-like HECT ubiquitin ligases (WWP1, WWP2, and Itchy), which regulate ubiquitination of viral Gag proteins and facilitate viral release. The endosomal DUB-associated molecule with the SH3 domain of STAM (AMSH) can deubiquitinate Gag proteins, although its role in viral egress remains unclear. The Gag protein of HIV-1 recruits the ESCRT proteins Tsg101 and ALIX and the E3 ligase NEDD4L for successful viral budding (58, 91).

4.5. Ubiquitination of Receptors for Endocytic Entry of Viruses

Virus entry via the endocytic pathway often requires ubiquitination of receptors. Dengue virus utilizes T cell immunoglobulin and mucin family receptor 1 (TIM-1) for its uptake. Ubiquitination of TIM-1 at Lys-338 and Lys-346 is needed to facilitate viral entry (15). Ubiquitination of the membrane receptor Nectin-1 by the E3 ligase Cbl is needed for internalization of HSV-1 and Kaposi's sarcoma–associated herpesvirus (17). IAV entry and uncoating needs the host Itchy ubiquitin protein ligase (ITCH), which promotes IAV escape from late endosomes via ubiquitination of the viral M1 protein (92).

5. UBIQUITIN-MEDIATED REGULATION OF CYTOSKELETAL DYNAMICS

The cytoskeleton has a fundamental role in regulating cell shape, motility, division, and intracellular transport of cargo. The cytoskeleton in vertebrates consists of four components: actin, microtubules, intermediate filaments, and septins. Dynamic actin polymerization is important for maintenance of cell shape and motility—intermediate filaments provide strength and plasticity; microtubules regulate interorganellar contact sites and intracellular transport of organelles (62) and protein complexes; and septins are involved in the formation of higher-order structures such as filaments, rings, and cage-like complexes in the cell. Upon pathogen entry, the cytoskeleton undergoes major changes to mobilize an antipathogenic response.

5.1. Actin Remodeling in Pathogenic Infections

Phagocytic uptake of the pathogen, as well as formation and stabilization of pathogen-containing vacuoles, causes changes in actin stress fibers. Some bacteria acquire actin tails for motility (examples include *Listeria* and *Rickettsia*). Septin cages around cytoplasmic bacteria have been observed in cells infected with *Shigella flexneri* and *Mycobacterium marinum*, which restricts their actin-based motility and targets them for autophagy (61).

Rho GTPases Rac1, RhoA, and Cdc42 are important regulators of actin dynamics. They are modulated by posttranslational modifications including phosphorylation, ubiquitination, and SUMOylation. Several E3 ligases, such as SMAD ubiquitination regulatory factor1 (Smurf1), SKP1-CUL1-F-box (SCF)^{FBXL19}, HECT domain and ankyrin repeat–containing E3 ubiquitin ligase1 (HACE1), and X-linked inhibitor of apoptosis and cellular IAP1 (XIAP), ubiquitinate Rho GTPases, ensuring their rapid turnover in response to different stimuli. Several bacterial effector proteins target the Rho family of GTPases, causing changes in actin dynamics at the plasma membrane to facilitate bacterial uptake. These effectors include SopE and SptP (*S. enterica*), IpgB2 and OspB (*S. flexneri*), TcdB (*Clostridium difficile*), VopS (*Vibrio parahaemolyticus*), and YpkO (*Yersinia enterocolitica*). In addition, during the first hour of infection, several proteins involved

in regulation of the actin cytoskeleton are ubiquitinated (23). How ubiquitination of these proteins affects actin polymerization and bacterial proliferation is not yet understood and will be interesting to study. *Salmonella* uses the host cell ubiquitination system to activate T3SS1 effector proteins SopE and SptP sequentially. This modulates Cdc42 activity and creates membrane ruffles needed for phagocytic uptake of bacteria. The host cell senses these cytoskeletal changes and activates innate immune signaling via activation of NF-κB and the inflammasome (18). The *Yersinia* effector YpkO interacts with the host DUB Otubain1 (OTUB1), which in turn regulates RhoA levels in the infected cell (20). Cortical protrusions of actin, such as lamellipodia and filopodia, are regulated by Cbl-mediated ubiquitination of the actin regulators mDab1 (mouse disabled homologue 1) and WAVE2, which inhibits formation of filopodia and lamellipodia, respectively. Recently, the E3 ligase $CUL3^{KCTD10}$ was reported to be important to dissolve actin bundles and bring about cell fusion in myoblast cells (79). It will be interesting to see whether similar actin-remodeling mechanisms are responsible for making protrusions that are used by bacteria such as *Shigella* to move from one cell to another. *L. monocytogenes* has an effector protein called internalin (InlA) that hijacks E-cadherin junctions in intestinal villus cells. Binding of InlA to E-cadherin causes its phosphorylation by Src tyrosine kinase and subsequent ubiquitination by the Cbl-like ligase Hakai, which triggers endocytic uptake of bacteria (25). Bacterial E3 ligases can also regulate cytoskeletal changes to promote infection. Enterohemorrhagic *Escherichia coli* has a HECT-like E3 ligase, NleL, that modulates formation of actin pedestals needed for bacterial infection (69). The E3 ligase of *S. flexneri* IpaH9.8 modifies human guanylate-binding protein 1 (hGBP1) by Lys-48-linked ubiquitin chains, causing its proteasomal degradation. Guanylate-binding proteins (GBPs) are host proteins that encapsulate bacteria, restricting their motility, and serve as scaffolds for assembling signaling complexes that cause oxidative killing of bacteria. Loss of hGBP1 enhances actin-based dissemination of bacteria (51, 98).

Virus entry and egress modulate the actin cytoskeleton, whereas intracellular assembly of virions utilizes microtubules. Virions often interact with the underlying actin cytoskeleton to gain entry into the cell. Viral entry requires high-affinity interactions with receptors that are associated with actin filaments inside the cell. Myosin motors that drive the actin cytoskeleton are responsible for pulling the receptor-virion complex across the plasma membrane. Entry of viruses through the endocytic pathway also requires remodeling of the cortical actin. For example, the entry process of HSV-1 triggers Cbl-mediated removal of Nectin-1, a process required for reducing the amount of membrane receptor needed for viral entry endocytosis. Cbl-depleted cells have a higher propensity toward viral entry and are more susceptible to secondary infections by HSV-1. The viral E3 ligase ICP0 interacts with CIN85, an adaptor protein that augments Cbl functions. As a result, in cells infected with the mutant ΔICP0 strain, Nectin-1 remains on the surface (17).

5.2. Microtubule Dynamics in Bacterial and Viral Infections

Bacterial effector proteins also target microtubules, either stabilizing or destabilizing them. Destabilization of microtubules benefits intracellular movement of pathogens and also allows remodeling of the actin network, through the activation of small GTPases. SifA is a *Salmonella* effector protein that is needed to form *Salmonella*-induced filaments (Sifs) that extend from SCVs. Sif formation requires dynamic microtubules. The *Salmonella* effectors SseG and SseF induce microtubule bundling (18). The *Brucella* effector TcpB stabilizes microtubules, which may hinder intracellular movement of organelles, thereby hindering phagosome-lysosome fusion (72). Tubulin is regulated by many types of posttranslational modifications in the cell, which determines microtubule stability and dynamics. These modifications include phosphorylation, acetylation, glutamylation, methylation, and ubiquitination. Ubiquitination of microtubules has been linked to formation of the mitotic spindle and flagellar motility (63, 101). Movement of

phagosomes along microtubule tracks also requires microtubule-associated motor proteins such as dynein and kinesins. These are also regulated by several E3 ligases such as PARKIN, SCF^{Fbxw5}, anaphase-promoting complex, and TRIM3 (83, 88).

Enveloped viruses interact with kinesins and microtubules that deliver packaged virions to the plasma membrane during viral egress. Here, virions engage myosins for transport across the cortical actin network for fusion at the plasma membrane and release into the synapse. In case of nonenveloped viruses, such as adenoviruses, viral protein VI is ubiquitinated by NEDD4, which ruptures the endosomal membrane and causes release of the virion into the cytosol. The E3 ligase Mib1 is needed for viral uncoating and release of the viral genome into the nucleus (82). For IAV, viral RNA segments are transported toward the nucleus on microtubule tracks and are released into the nuclear pore. The movement of viral RNA toward the nucleus is facilitated by the aggresome-processing machinery, HDAC6, unanchored ubiquitin chains, and molecular motors (5).

6. METABOLIC REPROGRAMMING DURING BACTERIAL AND VIRAL INFECTIONS

6.1. Glycolysis and Oxidative Phosphorylation

Mitochondrial changes are associated with reprogramming of cellular metabolism. The cell has two main programs to obtain energy from glucose: glycolysis and oxidative phosphorylation. During glycolysis, one molecule of glucose is converted to pyruvate in the cytosol. Under aerobic conditions, pyruvate is oxidized to carbon dioxide in mitochondria via the Krebs cycle, producing the reducing equivalents NADH and FADH2. These are fed to the electron transport chain, which pumps protons across the membrane to create an electrochemical gradient that enables the formation of ATP. In contrast, under anaerobic conditions, pyruvate is not fully oxidized but is fermented to lactate. Many proliferating cells, such as cancer cells, shuttle glucose toward glycolysis and lactate production, termed aerobic glycolysis or the Warburg effect. Though aerobic glycolysis is inefficient in terms of ATP yield, it can generate ATP much faster than ATP synthesis through mitochondrial respiration. Moreover, intermediates are redirected toward the biosynthesis of nucleotides, amino acids, and lipids needed in a highly proliferating cell. Pathogenic bacteria and viruses often reprogram host cell metabolism toward an aerobic glycolysis program. This redirects cellular resources toward the proliferating pathogen. Studies in *M. tuberculosis*–infected peripheral blood mononuclear cells showed transcriptional upregulation of genes related to glycolysis, which could be reversed by the mTORC1 (mechanistic target of rapamycin complex 1) inhibitor rapamycin (49). *Salmonella* uses the type III effector protein SopE2 to increase glycolysis and decrease serine synthesis, which is important for bacterial replication (38). *L. monocytogenes* alters fatty acid import into the mitochondria to regulate the balance between glycolysis and oxidative phosphorylation (16).

6.2. mTORC1 Is the Central Hub of Metabolic Pathways

Ubiquitination can regulate metabolic reprogramming by targeting several signaling hubs that modulate metabolism (**Figure 4**). The most important of these is mTORC1. Under nutrient-rich conditions, mTORC1 is active and supports growth and anabolic processes. The phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway also activates mTORC1 downstream of growth factor signaling. Low levels of amino acids or ATP lead to mTORC1 inhibition, shifting the metabolic balance toward catabolism and activation of autophagy. mTORC1 is inhibited by 5′ AMP–activated protein kinase (AMPK), which senses energy shortage in the cell. Members of the mTORC1 complex (mTOR, Raptor, DEPTOR, PRAS40, and Lst8), its direct regulators (RagA, GATOR, Rheb, TSC1, TSC2, and AMPK), and associated pathways are modulated by

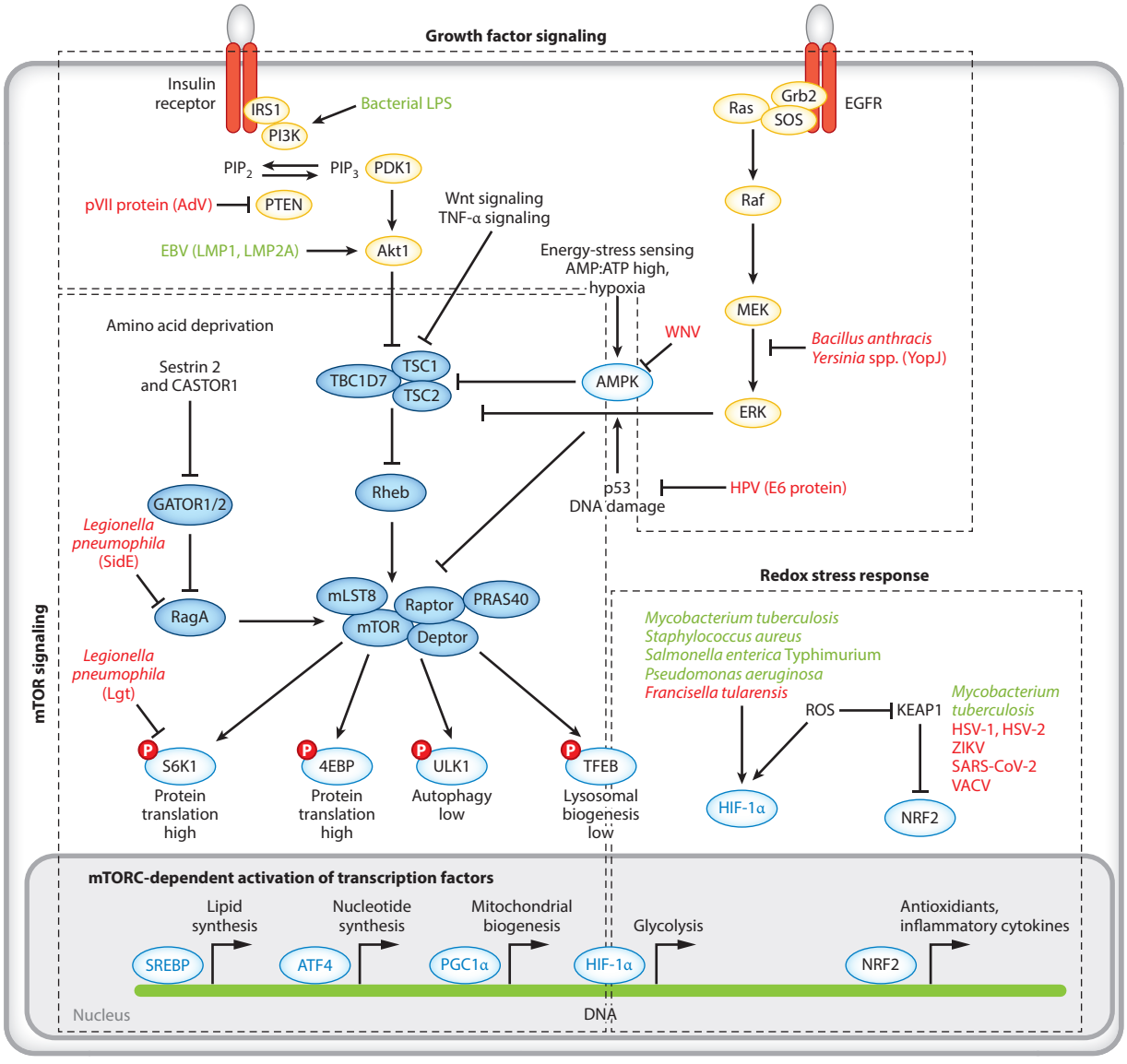

Figure 4

Regulation of metabolic pathways by bacterial and viral proteins. Growth factor signaling (e.g., insulin receptor, EGFR) can regulate metabolism through the control of mTOR, which is considered the central hub of metabolic pathways in the cell. mTOR senses amino acid levels in the cell and modulates the balance between anabolism and catabolism. Activation of mTOR signaling induces protein translation and causes transcriptional upregulation of genes that are important in anabolic processes (lipid synthesis, nucleotide synthesis, mitochondrial biogenesis, etc.). Redox sensors in the cell also affect metabolism through transcriptional regulation, as seen in the case of HIF-1α and NRF2. Pathogens and effectors that positively regulate the host protein are labeled in green; those with an inhibitory effect are labeled in red. Abbreviations: AMPK, 5′ AMP–activated protein kinase; EBV, Epstein-Barr virus; EGFR, epidermal growth factor receptor; HPV, human papilloma virus; HSV-1, herpes simplex virus 1; KEAP1, Kelch-like ECH-associated protein 1; LMP1, latent membrane protein 1; mTOR, mechanistic target of rapamycin; mTORC, mTOR complex; PI3K, phosphatidylinositol 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VACV, vaccinia virus; WNV, West Nile virus; ZIKV, Zika virus.

many E3 ligases and DUBs in the cell. Ubiquitination of mTOR-inhibitory proteins can cause their degradation, which in turn leads to activation of the pathway. For example, the stability of the TSC complex is regulated by several E3 ligases, including TRIM31, HERC1, Fbw5, and Pam. Proteasomal degradation of TSC complex components leads to activation of mTOR signaling. In some cases, ubiquitination modulates protein function; for example, Lys-63-linked ubiquitination of RagA by RNF152 activates mTOR by localizing RagA to the lysosome, whereas its ubiquitination by SKP2 recruits GATOR to turn off mTORC1 signaling (39). Aberrant mTORC1 signaling is considered a key feature of metabolic reprogramming in bacterial infection. The *Legionella* SidE ligases ubiquitinate and inactivate RagA, leading to deactivation of mTORC1. This inhibits protein translation and increases the availability of free amino acids for bacterial proliferation (13, 36). The YopJ effector of *Yersinia* is a cysteine protease that can cleave ubiquitin from substrates. It can regulate host cell metabolism indirectly through its inhibitory effect on the MAPK pathway (94).

Viruses hijack host metabolism to funnel resources toward the production of virions and to build specialized compartments for their own replication, maturation, and dissemination. Viruses can activate and/or inhibit the mTORC1 pathway, depending on the type of the virus, the stage of infection, and the type of infected cell (50). Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) and LMP2A can activate mTOR signaling via the PI3K-Akt axis. This promotes transformation of EBV-infected cells. The capsid protein of West Nile virus (WNV) interacts with AMPK, leading to its ubiquitination and degradation. This leads to a loss of AMP sensing by the mTOR pathway. Further, the loss of AMPK inhibits autophagy and contributes to accumulation of ubiquitinated protein aggregates that cause neuronal cell death in brain tissue of WNV-infected mice (46). The histone-like core protein VII p53 of human adenoviruses promotes self-ubiquitination and degradation of the E3 ligase MKRN1, which ubiquitinates and degrades phosphatase and tensin homolog (PTEN), a negative regulator of PI3K signaling. Activating PI3K-Akt signaling slows down apoptosis and prolongs viral replication in both acute and persistent infection (35).

6.3. Transcriptional Switches That Shift Metabolism in Favor of Pathogens

Metabolism is also rewired through transcriptional programs, such as those mediated by hypoxia-inducible factor 1α (HIF-1α), nuclear factor erythroid 2–related factor 2 (Nrf2), c-Myc, and p53 (45). HIF-1α-dependent gene expression can also lead to expression of genes involved in glycolysis [including hexokinase 1 (HK1), HK2, lactate dehydrogenase A, and pyruvate dehydrogenase kinase isoform 1 (PDK1)]. The stability of HIF-1α is regulated by the von Hippel–Lindau (VHL) tumor suppressor protein, which is a substrate-recognition component of an E3 ubiquitin ligase that targets prolyl-hydroxylated HIF-1α for degradation. Under hypoxic conditions, HIF-1α remains unmodified by prolyl hydroxylases and thereby escapes recognition by VHL and destruction. The stable HIF-1α dimerizes with HIF-1β to initiate the transcription of hypoxia-inducible genes. Loss of VHL in bone marrow–derived macrophages increases HIF-1α-dependent gene expression and intracellular killing of GAS and of *P. aeruginosa* (67). Other bacteria, such as *Francisella tularensis*, downregulate HIF-1α in the primary macrophages to prevent the shift to aerobic glycolysis. This metabolic reprogramming of the host cells is required for optimal intracellular replication of *F. tularensis* (104). In this case, activation of Atg5-independent autophagy in *F. tularensis*–infected cells supports bacterial replication by providing additional nutrients to bacteria (90). *Salmonella* also activates HIF-1 signaling by its siderophore salmochelin, independent of cellular hypoxia (29).

Alveolar macrophages infected with *M. tuberculosis* show an increased expression of antioxidant genes that is regulated by NRF2. At homeostasis, NRF2 is maintained in an inactive state in the

cytosol by association with its inhibitor protein KEAP1 (Kelch-like ECH-associated protein 1), which targets NRF2 for proteasomal degradation. In response to oxidative stress, KEAP1 is inactivated and NRF2 is released to induce NRF2-responsive genes. Upregulation of NRF2-dependent antioxidant gene expression favors bacterial survival in the *M. tuberculosis*–infected lung (80). Some viruses, including HSV-1 and HSV-2, vaccina virus, Zika virus, and SARS-CoV-2, downregulate NRF2-dependent gene expression, thereby reducing release of proinflammatory cytokines (66).

p53 can regulate transcription of several genes involved in glucose metabolism; for example, it can stimulate TP53-induced glycolysis and apoptosis regulator (TIGAR) and hexokinase and inhibit phosphoglycerate mutase (PGM), glucose transporter 1 (GLUT1), and GLUT4. At basal levels of p53, these effects inhibit glycolysis. The E6 protein of human papilloma virus (HPV) hijacks the host E3 ligase E6AP to cause degradation of p53, which is a tumor suppressor. Loss of p53 shifts energy metabolism toward aerobic glycolysis in HPV-infected cells (24). In summary, both bacteria and viruses reprogram metabolism in similar ways as cancer cells to hijack cellular energy resources for their own proliferation.

7. TARGETING UBIQUITIN-DEPENDENT SIGNALING IN INFECTION

Since ubiquitin-dependent processes are important in bacterial infection, their manipulation by drugs might be an effective therapeutic strategy. Molecules of the ubiquitin-proteasomal system have been targeted successfully in cancer therapy by proteasomal inhibitors such as bortezomib, carfilzomib, and ixazomib. The E1 enzyme inhibitor TAK-243 is also in clinical trials (56). However, in bacterial infections, only a few molecules of the ubiquitin pathway have been targeted by pharmaceutical reagents. The chemical compound oxathiazol-2-one preferentially inhibits the proteasomes of *M. tuberculosis* and can be used to treat *M. tuberculosis* infection. In cultured cells, the DUB inhibitor WP1130 (also called degrasyn) induced iNOS (inducible NO synthase)-dependent killing of *L. monocytogenes* in phagosomes (9).

The new drug discovery paradigm is centered around the concept of targeted protein degradation, where disease-causing molecules in the cell are selectively targeted for degradation by the cell's own proteolytic machinery. Proteolysis-targeting chimaeras (PROTACs) are bifunctional molecules in which one end of the drug binds the target and the other end binds an E3 ligase that ubiquitinates the target, directing it for proteasomal degradation (33). A recent study utilized this approach to conjugate the reversible inhibitor of HCV, an NS3/4A protease (telaprevir), to a cereblon-recruiting ligand. An optimized degrader, DGY-08–097, potently inhibited HCV in a cellular infection model, and protein degradation contributed to antiviral activity (14). Gram-positive bacteria, including mycobacteria, utilize the ClpC:ClpP (ClpCP) protease for targeted protein degradation. In this case, phosphorylated arginine residues on proteins serve as a degradation signal that is recognized by the ClpCP protease, causing proteolytic degradation of the target. Small-molecule degraders that have been developed based on this system are called BacPROTACs. They consist of a phosphorylated arginine–containing chemical adaptor that tethers substrates to the ClpCNTD receptor domain, targeting it for proteolytic degradation. BacPROTACs have been used to cause degradation of the model substrates in *Mycobacterium*. In the future, the ClpC-directed degraders may be applied to target gram-positive pathogens, such as *S. aureus* and *M. tuberculosis*, which are a serious global health concern (60).

8. CONCLUSIONS

During infection, bacteria and viruses have a common goal of overpowering the host defenses, as well as to hijack host resources to promote pathogen survival and replication. Through evolution, pathogens have acquired a complex set of molecular weapons that are collectively responsible for

the virulence of the pathogen. The secretion or activation of these virulence factors or effector proteins is regulated in a complex manner and varies depending on the cell type, the stage of infection, and the metabolic state of the host cell. The host cell also has a defense program to resist pathogenic attack. This is often called cell-autonomous immunity and is the first line of defense that comes into play before the immune system is activated. Host immune tactics at the single-cell level have a very great effect on disease progression and host survival. Ubiquitination is often used as a signal to reprogram signaling networks and metabolic processes upon pathogen entry. Through horizontal gene transfer, bacteria and viruses have acquired genes encoding proteins that modulate host ubiquitin and ubiquitin-dependent processes. A deeper understanding of this ubiquitin-mediated interplay is necessary to develop therapeutic approaches to combat infectious disease.

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AUTHOR CONTRIBUTIONS

R.M. and I.D. designed the structure for the review and wrote the manuscript. R.M. prepared the figures.

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