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REVIEW

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Taking control: Hijacking of Rab GTPases by intracellular bacterial pathogens

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ABSTRACT

Intracellular bacterial pathogens survive and replicate within specialized eukaryotic cell organelles. To establish their intracellular niches these pathogens have adopted sophisticated strategies to control intracellular membrane trafficking. Since Rab-family GTPases are critical regulators of endocytic and secretory membrane trafficking events, many intracellular pathogens have evolved specific mechanisms to modulate or hijack Rab GTPases dynamics and trafficking functions. One such strategy is the delivery of bacterial effectors through specialized machines to specifically target Rab GTPases. Some of these effectors functionally mimic host proteins that regulate the Rab GTP cycle, while others regulate Rabs proteins through their post-translation modifications or proteolysis. In this review, we examine how the localization and function of Rab-family GTPases are altered during infection with 3 well-studied intracellular bacterial pathogens, *Mycobacterium tuberculosis, Salmonella enterica* and *Legionella pneumophila*. We also discuss recent findings about specific mechanisms by which these intracellular pathogens target this protein family.

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Introduction

Intracellular bacterial pathogens are able to survive and replicate in their host cells by establishing an intracellular niche. Although many of these pathogens are facultative intracellular pathogens, and therefore can replicate both outside and within host cells, it is clear that they gain an advantage from living within specialized membranous compartments in the cytoplasm of the eukaryotic cell. This advantage often derives from increased access to nutrients or avoidance of the immune system. In contrast to non-pathogenic bacteria that are internalized and efficiently killed by phagocytic cells, intracellular pathogens survive and often replicate after internalization into eukaryotic cells. Upon phagocytosis, non-pathogenic bacteria are internalized into a compartment, the early phagosome, which is originated through the invagination of the plasma membrane. This compartment undergoes rapid maturation and, through a series of membrane trafficking events, matures into a late phagosome and finally into a phagolysosome, where the internalized bacteria are destroyed.[1,2] As it will be discussed below, bacterial pathogens are able to escape this fate, in most cases by subverting the trafficking mechanisms controlling this maturation pathway.

Rab GTPases are the largest group of the Ras superfamily of small GTPases, with more than 60 members encoded within the human genome. They regulate different intracellular membrane trafficking events, including membrane fission from donor compartments, membrane cargo transport along the cytoskeleton, and membrane tethering and fusion to acceptor compartments.[3,4] Many intracellular bacterial pathogens evolved strategies to specifically target these proteins to modulate these different trafficking events. In this review we will focus on 3 bacterial pathogens – *Mycobacterium tuberculosis, Salmonella enterica, Legionella pneumophila* – that have been shown to disrupt Rab GTPase localization or function by different strategies.

Mycobacterium tuberculosis *manipulation of the endocytic pathway*

The genus *Mycobacterium* includes many important intracellular bacterial pathogens. *Mycobacterium tuberculosis* (*M. tuberculosis*) is the cause of tuberculosis, a bacterial air-borne infection that affects around 9 millions people worldwide. *M. tuberculosis* is grouped with other genetically related bacteria that cause similar disease in other animals, forming the *Mycobacterium tuberculosis*

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complex. Alveolar macrophages are among the first cell types encountered by these bacteria in the lungs and, therefore, subversion of macrophage function is critical for establishment of an infection. The interaction of *M. tuberculosis* and the other closely related pathogens, including the attenuated strain *Mycobacterium bovis* (*M. bovis*) BCG, with macrophages has been extensively investigated and no significant difference in the ability of the 2 pathogens to interact with trafficking pathways in these cells has been reported. Therefore, we will use the term *Mycobacterium* in this review to refer without distinction to either *M. tuberculosis* or *M. bovis* BCG. It is well established that after phagocytosis *Mycobacterium* can survive within macrophages,[5] and that intracellular survival depends on the pathogen's ability to inhibit phagosome fusion with lysosomal content (Fig. 1).[6,7] The Rab GTPases Rab5 and Rab7 sequentially control the formation and subsequent maturation of the phagosomes into phagolysosomes.[1] It has been shown that while Rab5 is detected on the *Mycobacterium* phagosome, Rab7 is not, suggesting that this pathogen blocks the progressions of the phagosome through the canonical phagocytic pathway, by blocking the Rab5 to Rab7 step of maturation (also known as Rab5-Rab7 conversion).[8-10] Consistent with this hypothesis, both mature lysosomal hydrolases and the vacuolar ATPase are not detected on

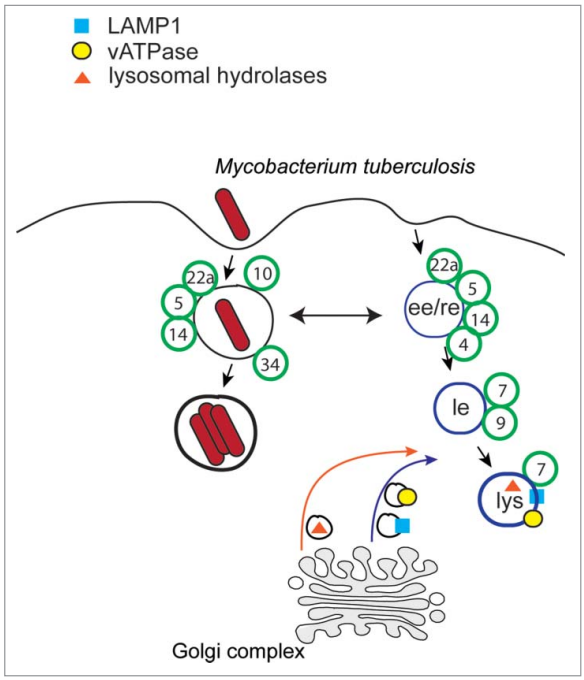

Figure 1. Trafficking model of the *Mycobacterium*-containing vacuole. After phagocytosis the *Mycobacterium*-containing vacuole acquires early-phagocytic features and Rab GTPases (green circles). However, it does not interact with the late endocytic pathway and does not acquire lysosomal markers, such as lysosomal hydrolases, the vATPase and lysosomal glycoproteins.

the *Mycobacterium*-containing vacuole.[11,12] The specific mechanisms by which *Mycobacterium* prevents phagosomal maturation are incompletely understood but it is clear that unique lipids of its envelope, such as the mannose-capped lipoarabinomannan (man-LAM), play a central role in this process. It has been shown that in contrast to control latex beads, man-LAM coated beads prevent the recruitment of the Rab5 effector early endosome antigen-1 (EEA1) and delivery of lysosomal enzymes to the phagosome.[9,13] This block of phagosome maturation was suggested to be mediated by an inhibition of the phosphatidylinositol 3-phosphate (PI3P) production by the PI3Kinase hVps34.[13] More recently, somehow in contrast with previous findings, it has been reported that *Mycobacterium* does not necessarily inhibit the Rab7 recruitment to the vacuole but, rather, it appears to inhibit its function.[14] In fact, it was shown that Rab7 is present in a GDP-bound form in *Mycobacterium*-infected macrophages and that mycobacterial infection inhibits the recruitment of the Rab effector, Rab-interacting lysosomal protein (RILP). In support of the idea that Rab7 is present but inactive on the mycobacterial phagosome, it has been reported that rate of fluorescence recovery for Rab7 on mycobacterial phagosomes is lower than that on the phagosomes containing latex beads.[15] More recently, the secreted mycobacterial nucleoside diphosphate kinase (Ndk; Table 1) was shown to act in vitro as a GTPase activating protein (GAP) for Rab5 and Rab7, suggesting a novel mechanism to prevents recruitment of RILP and EEA1 to the *Mycobacterium* phagosome.[16] However, the absence of Ndk has only a minor effect on the survival of *Mycobacterium*, suggesting that this bacterium may use additional strategies to prevent phagolysosome maturation.

In addition to Rab7, mycobacteria also interact with other endosomal Rab GTPases. For example, Rab34 is upregulated in *Mycobacterium*-infected macrophages[17] and Rab34 silencing or overexpression results in increased survival or killing of *Mycobacterium*.[18] Although the mechanisms by which Rab34 may limit *Mycobacterium* intracellular survival are not known, it is intriguing that RILP is also an effector for this GTPase. Furthermore, siRNA depletion of Rab34 impairs the fusion of phagosomes with late endosomes/lysosomes, while Rab34 overexpression promotes phagosomal maturation.[18]

Rab10 was also detected on the mycobacterial phagosome at very early time points after infection, even before Rab5 recruitment.[19] RNAi-mediated Rab10 knockdown or overexpression of Rab10 dominant-negative mutant delayed maturation of phagosomes of IgG-opsonized latex beads or heat killed-mycobacteria. Moreover, overexpression of a constitutively active mutant of Rab10 partially rescued live-*Mycobacterium*-containing

Table 1. Bacterial virulence factors, their biochemical activities, targets and effects on Rab GTPase function.

Viruence factor	Activity	Targets	Modulation of Rab function	Refs
Mycobacterium sp.				
Ndk	GAP	Rab5, Rab7	Deactivation	16
Salmonella enterica				
SopB	Phosphatidylinositide phosphatase	Phosphoinositides, Rho-family GTPases	Indirect	28-31
SifA	Binds SKIP, sequesters Rab9	SKIP	Rab9 sequestration	41,84
GtgE	Protease	Rab29, Rab32, Rab38	Removal	34,42
SopD2	Rab GAP	Rab32, Rab38	Deactivation	51
	Inhibitor of GEF activity	Rab7	Deactivation	56
				56
Legionella pneumophila				
VipD	Phospholipase A1	Rab5, Rab22	None	62
Lgp0393	GEF	Rab5, Rab21, Rab22	Unknown	63
DrrA or SidM	GEF, RabGDI	Rab1	Recruitment to the LCV	66-71
DrrA or SidM	Nucleotidyltransferase (AMPylation)	Rab1	Modulation of Rab1 recruitment to the LCV	72,73
AnkX	Phosphocholination	Rab1	Modulation of Rab1 recruitment to the LCV	74,75
SidD	DeAMPylation	Rab1	Modulation of Rab1 recruitment to the LCV	76-80
Lem3	Dephosphocholination	Rab1	Modulation of Rab1 recruitment to the LCV	76
LepB	Rab GAP	Rab1	Deactivation	81
SidE and SidE family effectors	NAD-dependent ubiquitination	Rab1, Rab33b	Unknown	83

phagosomes maturation.[19] These results suggested that Rab10 acts upstream of Rab5 to modulate the phagosome formation and maturation. It is not clear how Rab10 may modulate *Mycobacterium* intracellular survival. In response to insulin stimulation, this GTPase controls the translocation to the plasma membrane of the GLUT4 glucose receptor. However, it is not known if there is a link between this activity and the ability of this GTPase to modulate the intracellular replication of *Mycobacterium*.

Rab14 and Rab22a are also recruited to the mycobacterial phagosome.[20,21] Disruption of the function of Rab14 or Rab22a either by depletion or the expression of dominant negative mutants disrupts the maturation of the *Mycobacterium*-containing phagosome leading to the acquisition of lysosomal markers.[20,21] In this sense, the function of these GTPases appears to be opposite to the role of the GTPases discussed above. However, the specific role of these GTPases in the maturation of the *Mycobacterium*-containing vacuole is not understood.

In conclusion, many Rab GTPases can modulate the ability of mycobacteria to survive in macrophages, indicating a complex regulation of the trafficking events underpinning the phagocytic process and the phagolysosome formation. However, the mechanisms underlying the ability of mycobacteria to hijack these Rab GTPases or the specific roles that the different Rab GTPases play in the establishment of the *Mycobacterium* intracellular niche remains mostly unknown.

Salmonella enterica *interactions with endocytic and secretory Rabs*

Salmonella enterica (*Salmonella*) is an intracellular bacterial pathogen species that comprises more than 2,000 serovars. They cause a variety of illnesses in vertebrate hosts, ranging from self-limited intestinal infections to life-threatening diseases.[22] Furthermore, while the majority of *Salmonella* serovars can infect a broad range of hosts (e.g., *Salmonella* Typhimurium), others are extremely host-adapated (e.g., *Salmonella* Typhi).[18] Despite their different pathogenic behavior and host range, all the *Salmonella* serovars share a core set of virulence factors that allow them to enter and replicate within host cells. These properties are strictly dependent on the delivery of a set of bacterial effectors through 2 type III secretion systems (TTSS) encoded within their pathogenicity islands 1 (SPI-1) and 2 (SPI-2).[23,24]

Several studies have shown a close interaction between *Salmonella* and Rab-family GTPases. Immediately after *Salmonella* internalization, the *Salmonella*-containing vacuole (SCV) interacts with early endosomes and acquires Rab4, Rab5 and the Rab5 effector EEA1 (Fig. 2A).[25,26] Rab5 recruitment to the SCV and its retention are modulated by the SPI-1 TTSS effector protein SopB, which through its a phosphatidylinositide phosphatase activity modulates the phosphoinositide composition of the SCV.[27-31] Specifically, how the phosphoinositide composition of the SCV affects the retention of Rab5 is not understood.

Within 1 hour from bacterial internalization, the SCV recruits Rab7, and with it the Rab7 effector RILP and the lysosomal glycoproteins, such as LAMP-1 and LAMP-2.[25,32,33] The SCV also acquires the vacuolar proton pump V-ATPase responsible for the acidification of this compartment.[25] Rab7 function is essential for the vacuolar acquisition of the lysosomal glycoprotein LAMP-1 and for *Salmonella* replication in epithelial cells, since its depletion or the expression of dominant-negative forms of this GTPase result in significant reduction in

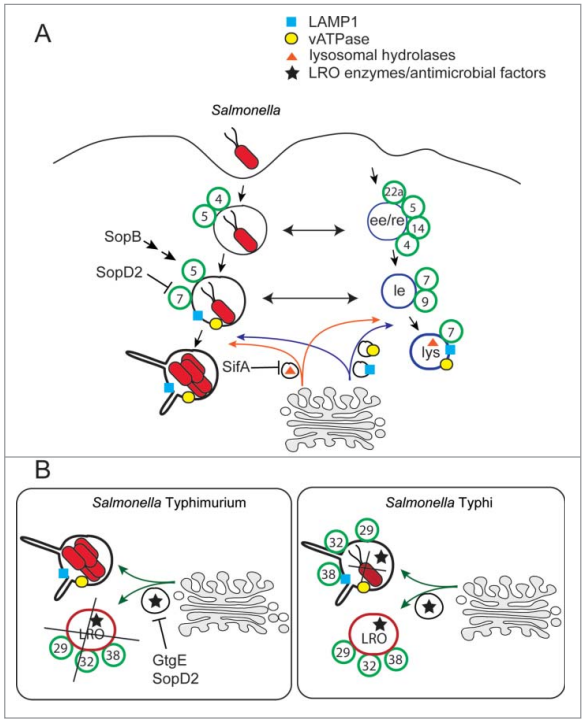

Figure 2. Trafficking model of the *Salmonella*-containing vacuole. (A) After phagocytosis the *Salmonella*-containing vacuole (SCV) acquires first early-endocytic features and later most of the lysosomal features. It also acquires sequentially early endocytic Rab GTPases and Rab7 (green circles). However, lysosomal hydrolases are not delivered to the SCV due to a SifA-mediated block of Rab9- and MPR-dependent transport pathway. Broad-host range *Salmonella* serovars, such as *S.* Typhimurium, target Rab32 and related Rab GTPases through GtgE and SopD2 and consequently inhibit the delivery of lysosome-related organelle (LRO) enzymes and antimicrobial factors to the LRO and SCV. (B) In contrast to the majority of other Salmonella serovars, the human-adapted *S.* Typhi does not deliver GtgE and SopD2 and, consequently, succumbs to the Rab32-dependent antimicrobial pathway in mice.

intracellular bacterial replication.[33,34] It has been shown that late endosomal/lysosomal content is transferred to the SCVs, early (30 min) after infection and continues for several hours, in a process that requires Rab7 and results in the acidification of the SCV.[35]

Therefore, in contrast to mycobacteria that replicates in an intracellular compartment devoid of lysosomal markers, *Salmonella* replicates in an intracellular niche that acquires many features of a lysosome, including acidic pH. Indeed, acidification of the vacuolar environment is essential for *Salmonella* survival and replication,[36] because it is thought to be a very important cue for the induction of the SPI-2 T3SS, which is critical for *Salmonella* survival and replication.[37,38] Although sharing many features with a lysosome, the SCV is still devoid of many properties characteristic of this compartment. For example, it never acquires the mannose-6-phosphate receptor (MPR) and enzymes that are transported to lysosomes in an MPR-dependent manner, such as cathepsin D.[39,40] It has been shown that the SPI-2 T3SS effector SifA interferes with the Rab9-mediated delivery of MPR to the SCV by sequestering this Rab GTPase in a complex with kinesin-interacting protein (SKIP).[41]

Recent work on the human-restricted pathogen *S.* Typhi has uncovered the existence of other Rab-dependent pathways leading from a post-Golgi compartment to the SCV. The Rab GTPases Rab32, Rab38 and Rab29, also known as Rab7L1, are recruited to the *S.* Typhi-containing vacuole, but not to the *S.* Typhimurium-containing vacuole (Fig. 2B).[34,42] All these 3 phylogenetically related Rabs localize on the Golgi or post-Golgi compartments.[34,43,44] Rab32 and Rab38 are known to be required for the post-Golgi trafficking of melanocytic enzymes to maturing melanosomes in pigment cells and specialized cargo to maturing dense granules in platelets.[43,45] Rab29, which is associated with risk of Parkinson disease, is involved in post-Golgi trafficking events that lead to neuronal development,[46,47] and is required for the transport of typhoid toxin from the *S.* Typhi vacuoles to the plasma membrane.[34] The absence of Rab32, Rab38 and Rab29 from the *S.* Typhimurium-containing vacuole is due to the activity of the T3SS effector protein GtgE, which is not present in the genome of *S.* Typhi and *Salmonella* Paratyphi, another human-restricted *Salmonella*. GtgE, which belongs to the clan CA superfamily of cysteine proteases, is a specific protease for these 3 Rab GTPases.[42,48,49] Remarkably, expression of GtgE in *S.* Typhi allowed this human-specific pathogen to overcome host-restriction and replicate in mouse tissues.[42] Further dissection of this phenotype indicated that Rab32 is the relevant target for GtgE to overcome this host restriction mechanism since removal of this GTPase or its guanine nucleotide exchange factor BLOC-3[50] allowed *S.* Typhi survival in mouse macrophages and replication in mouse tissues.[42] These studies therefore identified a novel Rab32-dependent pathogen restriction pathway that prevents the replication of the human-adapted *S.* Typhi and *S.* Paratyphi in non-human hosts.

The importance of this novel cell-intrinsic pathogen-restriction pathway is highlighted by the finding that *S.* Typhimurium targets this pathway in a functionally redundant manner. Indeed, in addition to GtgE, Rab32 is targeted by the T3SS effector protein SopD2.[51] This effector is widely distributed across *Salmonella* serovars but it is a pseudogene in the human-adapted *S.* Typhi and *Salmonella* Paratyphi serovars. SopD2 exerts its inhibitory function of Rab32 by acting as a GAP and stimulates Rab32 intrinsic GTPase activity.[51] SopD2

GAP activity results in the Rab32 displacement from the surface of the vacuole.[51] Although deletion of either GtgE or SopD2 does not eliminate the ability of *S.* Typhimurium to prevent Rab32 recruitment to the its vacuole, removal of both effectors results in efficient Rab32 recruitment. A *S.* Typhimurium strain defective for both SopD2 and GtgE is drastically impaired for its ability to cause a systemic infection in mouse.[51] However, this mutant strain is as virulent as a wild-type strain in Rab32- or BLOC-3-deficient mice. This indicates that *Salmonella* evolved redundant strategies to neutralize a critical Rab32-dependent host-defense pathway and establish a systemic infection. In melanocytes and in platelets Rab32 is involved in a pathway that delivers specialized cargo from post-Golgi compartments to maturing lysosomal-related organelles.[45,52] The role of Rab32 in post-Golgi trafficking suggests that in macrophages, and possibly in other cell types dedicated to host-defense, Rab32 controls a trafficking pathway delivering specialized molecules that can kill *S.* Typhi or other intracellular pathogens unable to neutralize this host-defense pathway.[42,53,54] Interestingly, Rab32 appears to have quite an opposite role in the intracellular growth of *L. pneumophila*, an intracellular bacterial pathogen that, as discussed below, hijacks the early secretory pathway to establish a replicative vacuole. Indeed, Rab32 is required for efficient *L. pneumophila* replication in lung carcinoma epithelial cells.[55] These observations highlight the substantial difference of survival strategies implemented by different bacterial pathogens.

It has been suggested that SopD2 may have an additional activity that allows it to interfere with Rab7 function. Indeed, it has been shown that SopD2 blocks endocytic traffic to lysosomes by binding Rab7 and acting as an inhibitor of the Rab7 guanine nucleotide exchange reaction through a poorly understood mechanism.[56] It has been reported that this inhibitory activity is dependent on its N-terminal domain, indicating that, whatever its mechanism, this inhibitory function must be independent of its GAP activity, which requires an arginine in the C-terminal end of the protein. Therefore, SopD2 seems to have evolved 2 functions to facilitate *Salmonella* survival in an intracellular compartment. One to prevent Rab7-mediated lysosomal fusion, and the other to neutralize an antimicrobial Rab32-dependent trafficking pathway.

In addition to Rab32, Rab38 and Rab29, other Rabs GTPases appear to also be excluded from the SCV. For example, Rab8B, Rab13, Rab23, Rab35 are enriched on model phagosomes, but are absent from the *S.* Typhimurium-containing vacuole.[57] Exclusion of these Rabs from the SCV appears to be dependent on the SPI-1 TTSS effector SopB.[31] In fact, a *S.* Typhimurium *ΔsopB* mutant shows recruitment of these 4 Rabs on its SCV. It has been reported that the phosphoinositide phosphatase SopB prevents the localization of these 4 Rab GTPases by reducing the level of negative charged lipids on the surface of SCV.[31] In conclusion, *Salmonella* has evolved multiple mechanisms to modulate or antagonize Rab GTPase function to create a compartment where this pathogen can survive and replicate.

Legionella pneumophila *subversion of secretory Rabs*

Legionella pneumophila (*L. pneumophila*) uses multiple complex strategies to interact with Rab GTPases. The natural hosts of *L. pneumophila* are fresh water amoebas and it is believed that this bacterium only occasionally causes human infections as the result of inhalation of aerosolized water droplets contaminated with *L. pneumophila*. It is thought that through extensive co-evolution with its natural host, *L. pneumophila* has evolved very sophisticated and most often redundant mechanisms to survive and replicate within the intracellular environment of the amoeba. The conservation of many basic cellular biologic processes dictates that many of the strategies evolved by *L. pneumophila* to thrive in its natural unicellular host also allow it to replicate within human macrophages (reviewed in ref. 58). Once internalized by human macrophages, *L. pneumophila* resides within a specialized compartment known as the *Legionella*-containing vacuole (LCV). The LCV does not follow the classical phagocytic maturation route and therefore does not fuse with lysosomes (Fig. 3). Rather, through the activity of multiple effector proteins of a type IV protein secretion system (T4SS), *Legionella* modulates membrane trafficking to build a specific phagosomal compartment (reviewed in refs. 59-61). The LCV does not acquire Rab5 or Rab7 indicating that it deviates from the canonical endocytic pathway pretty early after its formation. The mechanism by which *L. pneumophila* targets the early endocytic machinery are not known but it has been suggested that the effector protein VipD may contribute to this activity by binding Rab5 and Rab22, thus preventing their interaction with their downstream effectors Rabaptin-5 and EEA1.[62] Recently, another *L. pneumophila* T4SS effector, Lgp0393, was reported to target Rab5, as well as Rab21 and Rab22.[63] Lgp0393 is remotely related to the Rab5 guanine nucleotide exchange factor Rabex-5 and has a low guanine nucleotide exchange factor activity on Rab5, Rab21 and Rab22. The functional role of the interaction of Lgp0393 with endosomal Rab GTPases and this enzymatic activity still remain to be clarified.

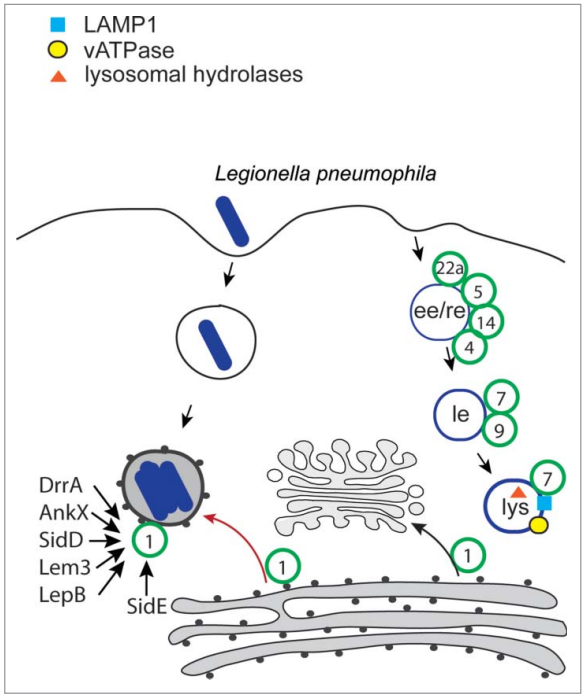

Figure 3. Trafficking model of the *Legionella*-containing vacuole. After phagocytosis the *Legionella*-containing vacuole (LCV) does not interact with the endocytic pathway and does not acquire any of endocytic Rab GTPases (green circles). However, it acquires the secretory Rab, Rab1, which is regulated and post-translationally modified by the Legionella T4SS effectors, DrrA, AnkX, SidD, Lem3, LepB and SidE.

Remarkably, at least 6 of the *L. pneumophila* T4SS effectors target the Rab GTPases Rab1 (reviewed in ref. 59). Rab1, a critical regulator of trafficking between ER and the Golgi complex, is recruited to the *Legionella*-containing vacuole within the first hour of infection.[64,65] An effector called DrrA or SidM acts as a guanine exchange factor (GEF) for this GTPase, resulting in tethering and fusion of endoplasmic reticulum derived vesicles to the LCV.[66,67] DrrA has a PI4P binding domain that mediates its interaction with the membrane[68] and a GEF domain with high affinity for the GDP-bound form of Rab1.[69-71] Because of its high affinity for the GDP-bound form of Rab1 DrrA function both as a GEF and a Rab-guanine nucleotide displacement inhibitor (RabGDI) displacement factor. In addition, the amino-terminal region of DrrA act as a nucleotidyl transferase that covalently attaches an AMP moiety onto a conserved tyrosine residue of Rab1 using ATP as a substrate, a reaction known as AMPylation.[72,73] In addition, to DrrA, L. pneumophila has evolved other effectors that modify Rab1 to stabilize it on the LCV and preventing its inactivation, indicating that controlling Rab1 activity is critical for L. pneumophila intracellular survival and replication. Another *L. pneumophila* effector, called AnkX, also modifies Rab1 through the addition of a phosphocholine (PC) moiety to a serine residue using CDP-choline as substrate, a reaction called phosphocholination.[74,75] Both the tyrosine residue AMPylated by DrrA and the serine residue phosphocholinated by AnkX are located within the switch II loop of Rab1, and, consequently, the modified Rab1 protein has reduced affinity for GAPs and effectors.[72,74] Remarkably, the AMPylation and phosphocholination modification on Rab1 are reversed by the concerted action of the type IV effector proteins SidD and Lem3, which respectively deAMPylate and dephosphocholinate Rab1 (reviewed in ref. 76). The deAMPylase reaction is performed by the effector protein SidD, an enzyme with structural similarity to metal-dependent protein phosphatases.[76-80] The activity of Rab1 is also regulated by the type IV effector protein LepB, a Rab GAP that is found associated to the LCV only later during infection, when the LCV has acquired endoplasmic reticulum features.[81] Therefore, Rab1 activity appears to be tightly controlled, likely to ensure the proper spatial and temporal activation of Rab1 in the *L. pneumophila*-infected cell to facilitate the proper sculpting of the LCV and its removal from the endo-lysosomal pathway. In addition to Rab1, some of the effectors described above can also target other Rab GTPases although the functional consequences of these interactions are unclear.[74,82]

L. pneumophila also targets Rab GTPases through ubiquitination mediated by a family of *L. pneumophila* effectors that use a novel mechanism of ubiquitination that does not require E2 or E3 ligases.[83] This family of effectors, which includes SidE, SdeA, SdeB, and SdeC, contains an aminoacid motif (R-S-ExE) found in mono-ADP ribosyltransferases.[83] This enzymatic domain mediates the ubiquitination of Rab33b, Rab1 and, to a lesser extent, other Rab GTPases associated with the endoplasmic reticulum and the Golgi complex, through a complex and unprecedented biochemical pathway, which involves the formation of a AMP-ubiquitin adduct and atypical direct transfer of ubiquitin.[83] Remarkably, Rab GTPases involved in pathogen internalization, such as Rab5, do not appear to be targets of this novel mechanism of ubiquitination. In summary, through mechanisms presumably evolved in the context of interaction with its natural unicellular host, *L. pneumophila* has adopted multiple mechanisms to modulate Rab GTPase function in macrophages and thus facilitate its intracellular survival and replication.

Conclusions

Intracellular bacterial pathogens have evolved multiple, often redundant mechanisms to target Rab GTPase proteins to modulate or antagonize their multiple and diverse functions. Remarkably, the 3 bacterial pathogens discussed in this review, *Mycobacterium tuberculosis,*

Salmonella enterica, and *Legionella pneumophila*, display unique trafficking subversion strategies to avoid intracellular killing. The study of these mechanisms has not only generated very important understanding of pathogenic mechanisms but has also provided truly unique insight into Rab GTPase function. Furthermore, some of these studies have revealed novel post-translational modifications of key regulatory proteins thus opening new vistas into eukaryotic regulatory mechanism. Remarkable as these discoveries have been, the fact remains that most of the activities of bacterial effector proteins are unknown, a clear indication that the best is yet to come.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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