Diverted recycling- Shigella subversion of Rabs

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Vesicular traffic, Rab subversion, Rab11, intracellular bacteria, bacterial containing vacuole, vacuolar rupture, *Shigella flexneri*, macropinocytosis.

Abbreviations

Bacterial containing vacuoles (BCVs).

Early endosome antigen 1 (EEA1).

Endoplasmic reticulum (ER).

Focused ion beam scanning electron microscopy (FIB/SEM).

GDP/GTP exchange factor (GEF).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

GTP-hydrolysis activating proteins (GAPs).

Kinesin superfamily proteins, kinesins (KIFs).

Lipoarabinomannan (LAM)

Lysosome-related organelles (LROs).

Mannose phosphate receptors (MPRs).

Multi-vesicular bodies (MVBs).

Myosin V (MyoV).

Phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3).

Phosphatidylinositol 3-phosphate (PI3P).

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2).

Phosphatidylinositol 5-phosphate (PI5P).

Pleckstrin homology domain-containing family M member 2 (SKIP).

Rab GDP dissociation inhibitors (GDIs).

Rab-interacting lysosomal protein (RILP).

Rab11 family-interacting protein (FIP).

Salmonella induced filaments (SIFs).

Trans-Golgi network (TGN).

Type-3 secretion system (T3SS).

Abstract

Small GTPases of the Rab protein family control intracellular vesicular trafficking to allow their communication and maintenance. It is a common strategy for intracellular bacteria to exploit these pathways to shape their respective niches for survival. The subversion of Rabs for the generation of an intracellular environment favoring the pathogen has been described almost exclusively for intracellular bacteria that reside within bacterial containing vacuoles (BCVs). However, less is known about Rab subversion for bacteria that rupture the BCV to reach the host cytoplasm. Here, we provide recent examples of Rab targeting by both groups of intracellular bacteria with a special focus on *Shigella*, the causative agent of bacillary dysentery. *Shigella* recruits Rab11, the hallmark of the perinuclear recycling compartment to *in situ* formed macropinosomes at the entry foci via the bacterial effector IpgD. This leads to efficient BCV rupture and cytosolic escape. We discuss the concept of diverted recycling through host Rab GTPases that emerges as a novel pathogen strategy.

Main text

Introduction

Intracellular bacteria have evolved molecular weapons to subvert host cells for their own benefit. They manipulate eukaryotic pathways for the promotion of efficient cellular invasion within a bacterial containing vacuole (BCV), intracellular survival, evasion of immune responses, and for their own propagation.[1] The bacterial weaponry that achieves such complex control is often constituted of an “effector cocktail”, secreted bacterial proteins into the host cytosol through specialized injection devices, like the type-3 secretion system (T3SS). These effectors interplay with host factors involved in the pathogenicity pathways. It has been recognized that the subversion of the vesicular trafficking machinery represents a key strategy by the bacteria in order to physically shape their local intracellular environment.[2-4]

Rab GTPases in vesicular trafficking

Cell membrane trafficking is regulated by the Rab family of proteins, which is part of the Ras superfamily of small GTPases. In humans, almost 70 different Rab proteins have been identified. They coordinate sequential trafficking steps by switching between their active GTP-bound form and the inactive GDP-bound form, participating at different levels for example during vesicle formation, motility, tethering or docking and fusion.[5]

Vesicles carrying internalized cargo from different entry pathways, such as clathrin and caveolae-mediated endocytosis, macropinocytosis and phagocytosis traffic to lysosomes for degradation. The pair Rab5-Rab7 is considered as the master regulator of endosomal trafficking and maturation. Rab5 determines the identity of early endosomal compartments.[6] One of its effectors, the phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3), produces phosphatidylinositol 3-phosphate (PI3P) on endosome

membranes, which in turn exerts a positive feedback loop resulting in increased Rab5 recruitment.[7] Rabex5, a GDP/GTP exchange factor (GEF) for Rab5, contributes to the positive loop by stabilizing Rab5 on the membranes.[8] More than 60 downstream effectors of Rab5 have been identified,[9] but their functions remain most often poorly understood. The progressive replacement of Rab5 by Rab7 is essential for proper maturation of early endosomes into lysosomes.[10-12] Upon Rab7 recruitment and activation, Rab5 is released allowing the maturation of early endosomes into late endosomes.[10] Retrograde transport of late endosomes along microtubules is necessary for efficient fusion with lysosomes. It is mediated by the binding of active Rab7 to Rab-interacting lysosomal protein (RILP), which induces the subsequent interaction with dynein-dynactin motor complexes.[13] In addition, the protein Hook has been shown to be involved in endosome maturation; its N-terminus interacts with microtubules and its C-terminus with membranes.[14]

Alternatively to catabolic degradation, and as a requirement for cell surface homeostasis, a part of the endosomal membrane and cargo are recycled back to the plasma membrane.[15] The membrane recycling system is highly dynamic; multiple pathways for entry and exit from the endocytic recycling compartment have been described with Rab11 present in most of them. Rab11 attracts effectors responsible for the trafficking of recycling endosomes.[16] It mediates vesicular transport along microfilaments by binding the globular tail domain of myosin V (MyoV).[17] Rab11 also interacts with the C-terminal of MyoV forming a ternary complex through Rab11 family-interacting protein 2 (FIP2).[18] In addition, Rab11 employs kinesins (KIFs) for anterograde transport along microtubules. FIP5 drives the interaction of Rab11 with KIF3.[19] Rab11 can bind directly KIF13 promoting the formation of tubules.[20] The effector protrudin directs the interactions of Rab11 with KIF5. Intriguingly, protrudin interacts selectively with GDP-Rab11[21]. This changes the concept of Rab11 being switched off in its GDP form, and could explain the

large promiscuity of Rab11 functions. FIP3 regulates the retrograde transport of Rab11 endosomes along microtubules mediated by dynein. This has been proposed for the sorting of peripheral endosomes to the perinuclear recycling compartment.[22] In addition, Rab11 is involved in the tethering and fusion of recycling endosomes with the plasma membrane [23,24].

Although Rab11 is the hallmark of the slow recycling pathway, its highly versatile nature and its complex localization profile in different cell types have led to numerous hypotheses about its roles. Importantly, only one Rab11 GEF, Crag, has been recently identified in *Drosophila*.[25] In mitosis, Rab11 has a role in organizing the mitotic spindle and spindle poles, and centriole distribution.[26] It is involved in ciliogenesis, cytokinesis, neuritogenesis, and oogenesis.[16] It controls the maintenance of microvilli and apical/basolateral specialization in epithelial cells,[27] and it is a regulator of autophagosome maturation.[28]

Besides the three Rabs, Rab5, Rab7 and Rab11, described in some detail above, other Rabs have been analyzed in the context of several local trafficking pathways. Exemplarily, Rab1 and Rab2 mediate endoplasmic reticulum (ER)-Golgi trafficking, Rab3 controls exocytic events, Rab32 is involved in the biogenesis of lysosome-related organelles (LROs) and mitochondrial fission, Rab6 regulates intra-Golgi trafficking, Rab9 and Rab7L1 mediate retrograde trafficking from late endosomes and lysosomes to the trans-Golgi network (TGN), Rab4 is involved in the fast recycling pathway, as has been reviewed in detail by Mizuno-Yamasaki and cols.[29] In addition, many of the Rabs have been described to be frequently involved in more than one single pathway.

Manipulation of Rabs by intracellular bacteria

Intracellular bacterial pathogens use different mechanisms to hijack Rab proteins (see **table 1** for an overview), especially those that reside within BCVs. They can mimic

Rab GEFs and GTP-hydrolysis activating proteins (GAPs) through their effector proteins, either favoring recruitment to or displacement of certain Rabs from their vacuoles. For instance, the phosphatase activity of the *Salmonella enterica* effector SopB is required for Rab5 recruitment to the BCV. SopB produces PI3P at the BCV membrane, which prolongs the association of Rab5 allowing BCV maturation. In addition, active Rab5 associates with the PI3KC3, which is responsible for augmented PI3P formation on the BCV membrane.[30] SopE, another *Salmonella* effector, has been found to act as a GEF for Rab5, recruiting non-prenylated Rab5 on the BCV.[31] *Mycobacterium tuberculosis* also interferes with Rab5. The glycolipid lipoarabinomannan (LAM), released from the bacterial membrane into the phagosomal membrane, inactivates the PI3KC3 impairing Rab5 recruitment.[32] Concomitantly, the *Mycobacterium* phosphatase SapM depletes PI3P contributing to the arrest of BCV maturation.[33]

Alternatively, some bacterial effectors bind Rabs and thus prevent subsequent interactions with their cognate host effectors. For instance, the *Legionella* effector VipD forms a complex with Rab5 and Rab22 preventing the interaction with their effectors, such as Rabaptin-5 and early endosome antigen 1 (EEA1).[34] This leads to a generalized impairment in endosomal maturation and, consequently, in pathogen degradation.[35] Another example is the complex formed by the *Salmonella* effector SifA and host protein pleckstrin homology domain-containing family M member 2 (SKIP). The SifA-SIKP complex induces the formation of *Salmonella* induced filaments (SIFs).[36] It also binds and recruits Rab9 to the BCV and SIFs. Sequestration of Rab9 by SifA-SKIP avoids the recycling of mannose phosphate receptors (MPRs), which prevents the delivery of lysosomal hydrolytic enzymes to the BCV and SIFs.[37] In addition, the C-terminus of SifA, which shows similar structure of other bacterial GEFs, binds RhoA although it does not change its GTPase status.[38] SIF formation is indirectly supported through another

175 *Salmonella* effector, SopD2, which binds directly to Rab7. This effector inhibits the
176 nucleotide exchange of this Rab. It impairs the recruitment of the Rab effectors to the BCV,
177 and subsequenly avoids degradation within lysosomes, thus allowing BCV maturation. [39]

178 Other bacterial effectors are able to covalently modify Rab proteins and
179 therefore completely change their properties. It has been described that the *Legionella*
180 effector DrrA, which is a GEF for Rab1,[40] covalently modifies Rab1 by AMPylation of its
181 Switch II region. Then, the AMPylated Rab1 restricts the access of GAPs, becoming
182 constitutively active at the BCV.[41] With opposed function, SidD reverses Rab1
183 AMPylation.[42] The effector LidA has also an auxiliary role on Rab1 recruitment thought the
184 action of DrrA,[43] and the effector LepB functions as a Rab1 GAP.[44] Another *Legionella*
185 effector named AnkX also covalently modifies Rab1. It transfers a phosphocoline group
186 from CDP-choline to a serine also in the Swich II region, leading to a strong inhibition of its
187 interaction with GEFs and Rab GDP dissociation inhibitors (GDIs).[45] Therefore, as
188 phosphocolinated Rab1 cannot be solubilized by the GDI, it remains membrane bound
189 even in the GDP form. The *Listeria monocytogenes* enzyme glyceraldehyde-3-phosphate
190 dehydrogenase (GAPDH) ADP-ribosylates Rab5. This covalent modification renders Rab5
191 unresponsive for activation by GEFs, and in turn it blocks further maturation into Rab7.[46]

192 Some effectors can even degrade Rabs by proteolytic cleavage. This is the case of
193 the *Salmonella* effector GtgE, which is expressed in the broad-host bacterium *Salmonella*
194 *enterica* serovar Typhimurium (*S.* Typhimurium) and helps to overcome the host restriction
195 barrier. This bacterial effector is specific for Rab32 and Rab7L1. In mouse macrophages,
196 GtgE avoids Rab32 recruitment to the BCV preventing delivery of antimicrobial molecules
197 to the BCV, and allowing bacterial survival and replication. Conversely, the human specific
198 serovars *S.* Typhi and *S.* Parathypi do not express GtgE. Rab32 is therefore recruited to
199 the BCVs, bringing antimicrobial capacity to the BCVs and killing intravacuolar bacteria.[47]

The *Salmonella* GAP for Rab32, SopD2, contributes to Rab32 removal from the BCV in S. Typhimurium.[48] However, in S. Typhi SopD2 is a pseudogene.[49] In addition, the absence of GtgE in the human-adapted *S.* Typhi and *S.* Parathypi allows Rab7L1 recruitment to the BCV, which is required for the export of typhoid toxin, a unique virulence factor for human-adapted serovars.[50]

The different examples of manipulation of Rab GTPases by pathogens described above have been characterized in some detail at the molecular level. However, this has not yet been achieved for many other cases. For example, the *Brucella abortus* effector RicA tethers Rab2 to its BCV by an unknown mechanism. *In vitro*, RicA binds preferentially GDP-Rab2 but does not possess GEF activity.[51] *Chlamydia trachomatis* recruits Rab6, Rab11 and Rab14 to the BCV in order to scavenge sphingomyelin from the Golgi.[52,53] It also recruits Rab39, which is involved in multi-vesicular bodies (MVBs) trafficking, for inclusion growth and bacterial development.[54] The specific bacterial effectors responsible of such subversions are still unknown. Finally, *Yersinia pestis* promotes the recruitment of Rab1 to the BCVs.[55] It is thought that this may be a mechanism to control vacuolar pH but, again, neither bacterial effectors responsible nor precise molecular events have been identified so far.

Subversion of Rabs by *Shigella*: Role in vacuolar rupture

For the causative agent of bacillary dysentery in humans, *Shigella flexneri*, Rab subversion has only been studied very recently. It has been shown that the *Shigella* effector VirA functions as a GAP for Rab1, mediating the disruption of ER-Golgi trafficking and suppressing host autophagy, which contributes to intracellular bacterial persistence.[56] Even though not directly acting on Rabs, IpaJ cleaves the Arf1 and Arf2 N-myristoylated host GTPases, which regulate Golgi trafficking, inhibiting thus the host secretory pathway.[57]

The scarce information about the usage of host Rabs by *Shigella* may be due to the intricate lifestyle of this pathogen. *Shigella* is a cytosolic bacterium that ruptures its BCV very rapidly after invasion,[58] replicates in the cytosol, and propagates into the neighboring cells.[59] The events occurring at the initial steps of infection are highly transient, making them difficult to be studied in detail.[60] In fact, in both reports mentioned above, the effects of the *Shigella* effectors were studied at late infection times (around 3 hours post-infection). At that stage bacteria are cytosolic and spread inter-cellularly. Therefore, the possible implication of VirA and IpaJ on Rab subversion at the early stages, such as bacterial entry and vacuolar escape, remains unclear.

As mentioned before, the step of vacuolar escape is a key event for *Shigella* virulence, but the implication of vesicular trafficking and Rab subversion in the process was never evaluated. Membrane damage, and thus vacuolar rupture, has been thought to occur mainly through the insertion of the T3SS injectisome on the BCV membrane.[61,62] Contrary to the established ideas, our group has demonstrated that BCV rupture requires host factors involved in vesicular trafficking, including Rab GTPases, to take place in an efficient manner.[63,64] In particular, *Shigella* hijacks Rab11 to newly formed macropinosomes leading to efficient BCV rupture. This relation was initially discovered characterizing data from a high-content siRNA library screen of host membrane trafficking factors involved in *Shigella* BCV rupture. BCV escape was monitored by automated microscopy using the CCF4 FRET probe as a reporter of vacuolar rupture.[65] This screen provided further Rab proteins to be potentially involved in vacuolar escape, such as Rab1, Rab3, Rab4, Rab5, Rab7L1 along with Rab11.[63]

Our live imaging revealed that Rab5, Rab7 and Rab11 were recruited to the *Shigella* entry sites upon bacterial invasion. While Rab5 was transiently recruited, Rab11 was massively and permanently accumulated.[63] Then, in order to evaluate the specific role

of Rab11 in vacuolar rupture, we used fluorescently tagged galectin-3 as live cell marker for loss of endomembrane integrity.[66] In Rab11 knockdown cells, *Shigella* vacuolar escape is dramatically delayed, but it does not affect bacterial entry. Importantly, the GTPase activity of Rab11 is necessary for efficient BCV rupture, since expression of GDP-locked [64] and GTP-locked Rab11 (non published results) led to a strong delay in vacuolar rupture. We found that Rab11 recruitment to the entry foci was entirely controlled by the bacterial effector IpgD,[63] an inositol phosphate phosphatase that converts phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) to phosphatidylinositol 5-phosphate (PI5P).[67] In agreement with its implication in Rab11 recruitment, the *Shigella* effector IpgD exhibited a strong delay in vacuolar rupture. In addition, we demonstrated that IpgD was also involved in the regulation of macropinosome formation during bacterial invasion. The IpgD mutant, which entered without any delay into epithelial cells, was dramatically impaired for macropinosome formation during the time of invasion.[63] Previously, IpgD, through the action of PI5P production was reported to be involved in the shaping of the entry foci.[68]

Canonical macropinocytosis has been described as clathrin-independent non-selective endocytosis (see **figure 1** for a comparison of *Shigella* induced macropinosomes). By macropinocytosis, cells are able to internalize considerable volumes of extracellular fluid for the uptake of soluble molecules as well as particles such as viruses, bacteria and apoptotic cell fragments. Antigen presenting cells are capable of constitutive macropinocytosis, while in macrophages, lymphocytes, fibroblast and epithelial cells macropinosomes appear upon applying a variety of external stimuli [69]. Macropinosomes are directed towards the lysosomal pathway though the Rab5/Rab7 cascade, Lamp1 acquisition and acidification.[70] The recycling of them has only been suggested by a few studies, furthermore the molecular mechanism of this and its regulation remains unclear. Intriguingly, in some non-professional phagocytic cells

macropinosomes have been reported to recycle to the plasma membrane, with little or no interaction with endosomal vesicles.[71] Our recent study on *Shigella* induced macropinosomes has been the first that proposed a direct implication of Rab11.

Classically, it has been considered that *Shigella* takes advantage of ruffling for its own internalization within macropinosome-like vesicles.[72] In contrast to this paradigm, our results from multidimensional live cell confocal microscopy showed that the recruited Rab5, Rab7 and Rab11 were located exclusively at the membranes of the surrounding macropinosomes (**figure 1**), but never at the forming BCV.[64] At the ultrastructural level, the different identities of both compartments was confirmed by focused ion beam scanning electron microscopy (FIB/SEM), an emerging technique for tomography of large volumes,[73] using a correlative approach. This showed clearly that *Shigella* enters in a tight vacuole distinct from the surrounding macropinosomes. In addition, FIB/SEM revealed the presence of contact sites between the BCV and macropinosomes, with the appearance of smaller intramacropinosomal vesicles right at their interface during vacuolar rupture.[64] Although Rab5 and Rab7 are recruited to *Shigella* induced macropinosomes, they do not recruit Lamp1 nor do they show acidification (non published results). Instead, they are blocked for further maturation and hijack Rab11 for virulence purposes. These observations suggest that BCV rupture may be mediated by direct physical contacts between Rab11-positive macropinosomes and the BCV. Such contacts may be an outcome of “misdirected” trafficking towards the BCV instead of their recycling to the plasma membrane, which could be considered as an “internal recycling” pathway.

In the scenario of “internal recycling” the exocyst (a protein complex made of eight distinct proteins) may play a role. Rab11-positive recycling endosomes are delivered to the apical cell surface along radial actin filaments by MyoV.[17] Then, they are tethered to the plasma membrane by binding of MyoV and Rab11 to the exocyst component Sec15 to

300 form a tripartite docking complex. Afterwards, Sec15 interacts with SNARE complexes to
301 mediate vesicle fusion with the plasma membrane.[23,24] Knocking down constituents of the
302 exocyst inhibited the process of *Shigella* internalization (our own unpublished
303 experiments), but the precise step of inhibition needs to be elucidated.

304 Since the GDP/GTP status of Rab11 is important for BCV destabilization, another
305 possible role for Rab11 in BCV rupture could be as a shuttle, providing the nascent
306 macropinosomes with downstream molecules involved in contact formation. It has been
307 described in *Drosophila* that under fed conditions the protein Hook has a negative
308 regulatory role in autophagosome maturation. Hook anchors late endosomes to
309 microtubules and thus impairs their fusion with amphisomes, delaying their maturation. In
310 this context Rab11 is prominently located at recycling endosomes. In starving conditions,
311 Rab11 regulates the relocalization of Hook protein in a GTP-dependent manner binding
312 and recruiting Hook to autophagic structures. Late endosomes are then not anchored
313 anymore to microtubules, and their fusion with amphisomes is favored, allowing a faster
314 maturation and catabolic degradation.[28]

315 Interestingly, Rab 11 has been also reported to be involved in expulsion of pore
316 forming toxins from the surface of challenged host cells.[74] A similar function has been
317 described for the ESCRT machinery, which is involved in local membrane deformation and
318 scission, and in repairing plasma membrane wounds.[75] It could be considered that Rab11
319 is recruited together with the ESCRT complex to the BCV via macropinosomes to repair
320 the damage caused by the insertion of the T3SS translocon complex, pinching off small
321 intraluminal vesicles at the contact interphase, and inducing in turn vacuolar rupture.

322 Finally, the notion that endosomal compartments function as multifunctional
323 platforms on which unique sets of molecular machines are assembled have emerged
324 recently.[76] Therefore, *Shigella* induced macropinosomes may represent a platform for

localized production of specific signalling lipids and docking of certain protein complexes promoting vacuolar scape.

Conclusion and perspective

In summary, as a result of Rab subversion, bacterial effectors induce or avoid multiple interactions between the BCV and other host cell compartments modifying the BCV or its membrane composition. Therefore, bacteria establish an intracellular replicative niche through the generation of a sort of hybrid organelle.[3,4]

Most of the currently described Rab modifications by pathogens lead to an impairment of BCV degradation for bacteria survival. This is achieved either by blocking phagosome maturation, by avoiding fusion with lysosomes or by redirecting BCV to alternative trafficking pathways such as the ER. In addition, intracellular bacteria also subvert vesicular trafficking in order to gain access to nutrients or scavenge building blocks for intracellular replication.

From our findings about the cytosolic bacterium *Shigella* we propose that the concept of Rab subversion is not an exclusive strategy for BCV-contained bacteria, but also for cytosolic ones. We have discovered a new role for recycling endosomes, via Rab11, during early stages of *Shigella* infection. This provides a different concept about bacterial vacuolar rupture. Intriguingly, none of the different Rabs recruited to the entry foci (Rab5, Rab7 and Rab11) were located on BCV membranes, but only on macropinosomes. Further research on Rab manipulation by cytosolic bacteria will help to fully understand the process of vacuolar rupture, a key event for their virulence.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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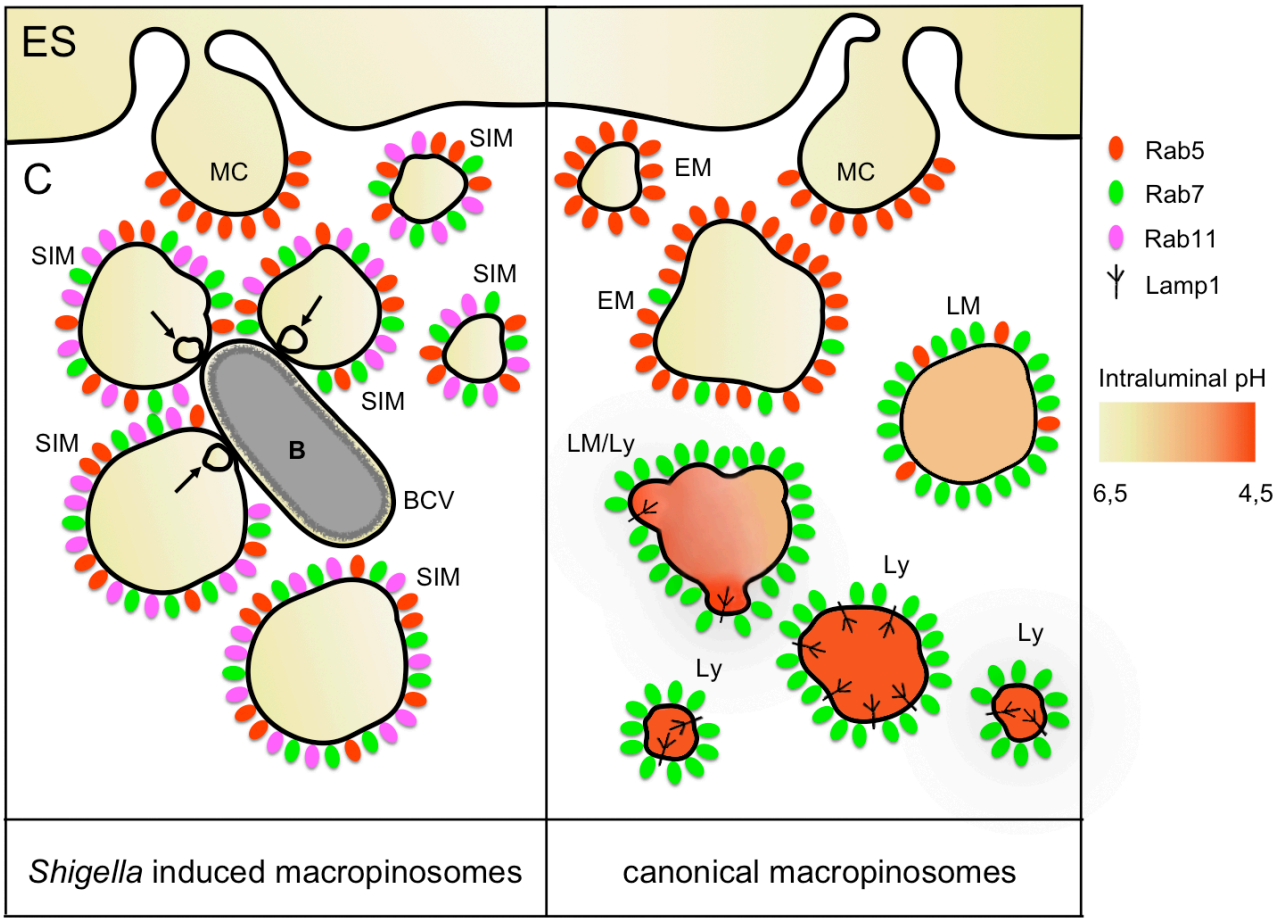
Pathogen	Effector	Mechanism of subversion	Effect on Rabs	Advantages for infection	References
Salmonella enterica	SopB	Inositol phosphate phosphatase. Converts PIP3 to PI3P	Prolongs and increases Rab5 recruitment on the BCV.	Delays interaction of BCVs with late endosomes	Mallo et al. (2008)
	SopE	GEF for Rab5. Acts on non-prenylated Rab5	Prolongs and increases Rab5 recruitment on the BCV.	Delays interaction of BCVs with late endosomes	Mukherjee et al. (2001)
	SifA	Forms a complex with SKIP, which binds Rab9	Induces recruitment of Rab9 to BCVs and SIFS	Prevents the delivery of hydrolytic enzymes to the BCV	Brumell et al. (2002) McGourty et al (2012)
Salmonella enterica sv. Typhimurium	GtgE	Rab32 protease	Avoids Rab32 recruitment to the BCV	Prevents the delivery of antimicrobial molecules to the BCV	Spanò and Galan (2012)
	SopD2	GAP for Rab32	Contributes to Rab32 removal from BCV membranes	Prevents the delivery of antimicrobial molecules to the BCV	Spanò et al. (2016)
		Binds to Rab7	Stabilizes Rab7 on SCVs and inhibits GTPase cycling.	Contributes to the evasion of lysosomal degradation, promotes the formation of SIFs	D'Costa et al. (2015)
Micobacterium tuberculosis	LAM	Inacivates 3PI3PKC3 inhibiting PI3P production	Impairs recruitment of Rab5 to the BCV	Arrests phagosome maturation and degradation	Fratti et al. (2001)
	SapM	Acid phosphatase. Dephosphorylates PI3P	Depletes PI3P on BCVs avoiding Rab5 recruitment	Arrests phagosome maturation and degradation	Vergne et al. (2005)
Legionella pneumophila	VipD	Binds to Rab5 and Rab22	Prevents interaction of Rab5 and Rab22 with their effectors	Decreases bacterial degradation by general impairment of endosomal maturation	Ku et al. (2012) Gaspar et al. (2014)
	DrrA	GEF for Rab1. Modifies Rab1 by AMPylation	AMPylated Rab1 is inert to GAPs and becomes constitutively active on BCVs	Establishment of a replicative niche with ER characteristics. Tight modulation of the different effectors is necessary to control BCV trafficking during the different phases of infection	Murata et al. (2006) Müller et al. (2010)
	SidD	DeAMPylates Rab1	Opposed function of DrrA		Tan and Luo (2011)
	LidA	Binds GDI-free Rab1 previously activated by DrrA	Involved in accumulating activated Rab1 on BCVs		Machner and Isberg (2007)
	LepB	GAP for Rab1	Deactivates Rab1 on the BCV		Gazdag et al. (2013)
	AnkX	Transfers phosphocholine to Rab1	Phosphocholinated Rab1 is inert to GEFs and GDIs on BCV membranes		Tan et al. (2011)
Listeria monocytogenes	GADPH	ADP-ribosylates Rab5	Recruits Rab5 to BCVs and inhibits the activation of GEFs	Blocks maturation of the phagosomes	Alvarez-Dominguez et al. (2008)
Brucella abortus	RicA	Binds to Rab2	Tethers Rab2 to the BCV	Helps to establish a replicative BCV with ER characteristics	de Barsy et al. (2011)
Chlamydia trachomatis	?	?	Recruitment of Rab6, Rab11, Rab14 and Rab39 to the BCV	Scavenge sphingomyelin for bacterial nutrition from Golgi and MVBs	Rejman Lipinski et al. (2009) Capmany and Damiani (2010) Gambarte Tudela et al. (2015)
Yersinia pestis	?	?	Recruitment of Rab1 to the BCV	Avoids acidification of BCVs to establish a replicative niche	Connor et al. (2015)
Shigella flexneri	VirA	GAP for Rab1	Deactivates Rab1 on Rab1 positive endosomes	Inhibits ER-to-Golgi transport and impairs autophagy	Dong et al. (2012)
	IpgD	Inositol phosphate phospatase. Converts PI(4,5)P to PI5P	Induces the recruitment of Rab11 to macropinosomes	Enhances vacuolar rupture and bacterial escape into the cytosol	Mellouk et al. (2014) Weiner et al. (2016)

568 **Table 1: Examples of Rab subversion by different intracellular bacterial pathogen** 24



569 **Figure 1: Schematic representation of macropinosome-like vesicles induced by**
570 ***Shigella* infection in comparison to the canonical macropinocytic pathway.** On the
571 right side, canonical macropinosomes traffic along the endolysosomal pathway, where they are
572 eventually degraded. Rab5 (red) is first recruited to nascent macropinosomes, similar to its
573 recruitment to early endosomes. Then, it is replaced by Rab7 (green). Acquisition of Rab7 implies
574 retrograde transport along microtubules and subsequent fusion with lysosomes. Upon fusion with
575 lysosomes, macropinosomes acquire lysosomal markers such as Lamp1 and hydrolytic enzymes
576 leading to their acidification. In contrast, macropinosome-like vesicles induced by *Shigella*
577 infection block their maturation before their fusion with lysosomes (left side). Rab11 (magenta) is
578 instead recruited by the bacterial effector IpgD. Bacterial subversion of Rab11, and its recruitment
579 to *Shigella*-induced macropinosomes, promotes efficient vacuolar escape. ES, extracellular space;
580 C, cytosol; MC, macropinocytic cup; SIM, *Shigella* induced macropinosome; BCV, bacteria
581 containing vacuole; B, bacteria; EM, early macropinosome; LM, late macropinosome; LM/Ly, late
582 macropinosome-lysosome; Ly, lysosome; arrow, intramacropinosomal vesicle.