

Functional role(s) of phagosomal Rab GTPases

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Rab GTPases are at the central node of the machinery that regulates trafficking of organelles, including phagosomes. Thanks to the unique combination of high quality phagosome purification with highly sensitive proteomic studies, the network of Rab proteins that are dynamically associated with phagosomes during the process of maturation of this organelle is relatively well known. Whereas the phagosomal functions of many of the Rab proteins associated with phagosomes are characterized, the role(s) of most of these trafficking regulators remains to be identified. In some cases, even when the function in the context of phagosome biology is described, phagosomal Rab proteins seem to have similar roles. This review summarizes the current knowledge about the identity and function of phagosomal Rab GTPases, with a particular emphasis on new evidence that clarify these seemingly overlapping Rab functions during phagosome maturation.

The Process of Phagosome Maturation

The dynamic and complex process of phagosome maturation is the result of multiple interactions between the phagosome and various intracellular compartments.^{1–3} Biological events happening in the lumen and membrane of phagosomes have a profound impact on the development of an appropriate innate and adaptive immune response.^{4,5} Compelling evidence from different studies using live cell imaging and proteomic analysis indicates that the classical view of phagosome progression as a linear pathway interacting sequentially with components of the endocytic pathway is a very simplistic view of this process.^{6,7} These studies have shown that phagosome maturation is a very dynamic process and all kinds of transient and rapid interactions occur simultaneously contributing to the maturation of the phagosome.^{7,8}

Rab GTPases

Rab proteins have emerged as central regulators of the dynamic process of interactions between phagosomes and intracellular compartments.² Rab GTPases are a large family of small GTPases that regulate intracellular transport.^{9,10} They operate at

different layers of regulation, determining the fusion partners, defining the lipid composition of the membrane via recruitment of specific enzymes, affecting the vesicle motility through molecular motors and modulating vesicular transport through interactions with cytoskeletal components.^{9,10} Therefore, once the Rab GTPases are localized in membranes, they define the biology of the compartment where they are located. Consequently, a particular Rab network will determine the precise biochemical composition and intracellular behavior of a compartment.⁹

Multiple Rab Proteins are Associated with Phagosomes

Facilitated by the availability of unique methodologies to purify phagosomes, their composition is relatively well described.^{7,8,11–14} Phagosome purification represents a powerful technique that allows the unambiguous biochemical description of proteins associated with a well-defined organelle.^{6,15} Thanks to the development and continued improvement of phagosome purification techniques, different groups have studied the recruitment of proteins, including Rab proteins, to phagosomes. These studies clearly showed that the process of phagosome maturation is highly dynamic even at a relatively short time after phagosome formation.^{7,8} In particular, phagosome purification techniques combined with proteomic studies have clearly shown that multiple Rab GTPases associate to particle-containing phagosomes (Table 1).

Assuming that the recruitment of specific proteins onto phagosomes is linked to particular functions required in this organelle, specific association of a defined group of Rab proteins would endow the phagosome with a distinct molecular behavior. Although the identities and functions of many phagosomal Rab proteins are relatively well known, their role(s) in the coordination of specific steps within the network of phagosomal Rab GTPases are still poorly defined.

The Network of Phagosomal Rab GTPases

Although there must be distinctions based on different conditions, protocols of phagosome purification, nature of the ligand used for internalization, methodology used for analysis, age of the analyzed phagosomes, activation status of the cell and obviously the cell type, there are still some common tendencies in the identity of phagosomal Rab proteins (Table 1). Based on the number of times that the Rab proteins were detected under different experimental and technical systems and the level of

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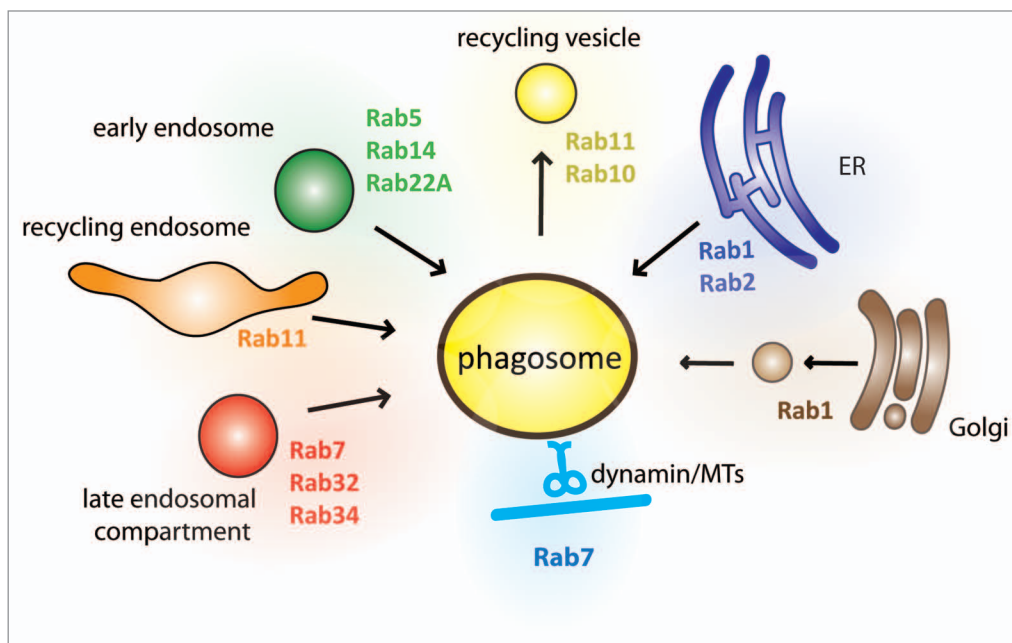


Figure 1. The network of phagosomal Rab GTPases: the most common Rab proteins. This model shows the functional link between Rab proteins associated with phagosomes, interactions with different intracellular compartments and/or cytoskeletal components. As discussed in the text, studies have shown that seemingly overlapping functions are in fact more specific. MTs, microtubules; ER, endoplasmic reticulum.

understanding of the function in phagosomes, phagosomal Rab GTPases can be generally divided into 2 groups: The first group contains the Rab proteins that are commonly detected in the proteomic studies discussed within this review (Fig. 1). These Rab proteins were identified using phagosomes containing various particles that can be unambiguously isolated and analyzed. The second group of Rab GTPases contains the Rab proteins that are less often identified in the proteomic studies (Fig. 2). This group includes phagosome-associated Rab proteins with unknown or poorly characterized phagosomal functions as well as Rab proteins not commonly identified as being associated with phagosomes but having a well-described phagosomal function.

Other studies have described Rab proteins associated with bacteria-containing phagosomes.¹⁶⁻¹⁸ For the sake of simplicity, these bacterial phagosome proteomes are not primarily considered here. To define the composition of bacteria-containing phagosomes is in general more difficult since their composition is very heterogeneous and the risk of contamination with other vesicles is very high. Moreover, intracellular pathogens can manipulate Rab functions increasing the complexity of the Rab proteins associated with different phagosomes, vacuoles, niches etc.¹⁹ Therefore, as discussed below, the presence of certain proteins in bacterial phagosomes could also reflect active manipulation by the bacteria.

Rab GTPases Commonly Identified in Phagosomes and with a Relatively Well-Assigned Phagosomal Function (Fig. 1)

Rab1

This GTPase is mainly related to the transport from the endoplasmic reticulum (ER) to the Golgi complex.²⁰ The isoform

Rab1A in mammalian and *Drosophila melanogaster* cells and Rab1D in *Dictyostelium discoideum* are recruited into particle-containing phagosomes.^{7,8,12-14} In cells infected with *Legionella pneumophila*, Rab1 is early and efficiently recruited in the *L. pneumophila*-containing vacuole.^{21,22} It has been demonstrated that *L. pneumophila* uses and manipulates Rab1 to survive within cells.²³⁻²⁶ *Legionella* hijacks the host cell machinery to receive components from the secretory pathway and creates an ER-like niche.²⁷ Although the manipulation of the Rab1 function by *Legionella* is the one best characterized for this bacterium, this is not the only pathogen that interacts with Rab1 positive vesicles from the early secretory pathway. Rab1 is also recruited into the acidic *Coxiella burnetii* containing vacuoles, and it is required for the growth of this intracellular pathogen.²⁸

However, the precise function of Rab1 during phagosome maturation is not well defined. It is possible that Rab1 could potentially be part of a formerly described Golgi to phagosome pathway that could deliver critical components necessary for phagosome maturation.²⁹⁻³¹ In another scenario, it could also be possible that Rab1 mediates the fusion between the ER and phagosomes^{32,33} and pathogens use this functional property to create a specific niche that fulfill their own requirements. Alternatively, Rab1 is a potential candidate that could regulate the contribution of the ER to phagocytosis.³⁴ Consistent with this notion is the existence of a *Legionella* effector that generates active Rab1, mediating ER-derived vesicles recruitment on the plasma membrane.³⁵

Rab2

Rab2 has been identified in many phagosomal proteomes arguing for an important role of this GTPase in phagosomes.^{8,11-14} Similar to Rab1, the small GTPase Rab2 is known to be located

Table 1. The phagosomal Rab GTPases. Rab GTPases associated with particle-containing phagosomes in 6 proteomic studies

	Garin et al.	Gotthardt et al.	Stuart LM et al.	Rogers and Forster	Jutras et al.	Trost et al.
Rab1	X	✓ (D)	✓	✓ (A/B)	✓	✓ (A/B)
Rab2	✓	X	✓ (A)	✓ (A)	✓	✓ (A)
Rab3	✓ (C)	X	X	X	X	✓ (D)
Rab4	✓	X	✓	X	X	✓ (B)
Rab5	✓	X	✓	✓	✓	✓
Rab6	X	X	✓ (A)	✓	X	✓ (A)
Rab7	✓	✓	✓	✓	✓	✓
Rab7B	X	X	X	✓	X	✓
Rab8	X	X	✓	✓ (A/B)	X	✓ (A/B)
Rab9	X	X	X	X	✓	✓
Rab10	✓	✓	✓	✓	✓	✓
Rab11	✓	✓ (A/C)	✓	✓ (B)	✓	✓ (A/B)
Rab12	X	X	X	X	X	✓
Rab14	✓	✓	✓	✓	✓	✓
Rab18	X	X	✓	✓	X	✓
Rab20	X	X	X	X	X	✓
Rab21	X	X	X	X	X	✓
Rab22A	X	X	X	✓	X	✓
Rab23	X	X	X	X	X	✓
Rab24	X	X	X	X	X	✓
Rab32	X	✓	X	✓	✓	✓
Rab34	X	X	X	✓	X	✓
Rab35	X	X	✓	✓	X	✓
Rab43	X	X	X	X	X	✓

Proteomes of bacteria-containing phagosomes are not considered here (see text). Letters in brackets refer to the specific isoform detected.

on secretory vesicles that traffic between the ER and the Golgi complex.³⁶ Mammalian Rab2 controls protein sorting and recycling from pre-Golgi intermediates.³⁶ UNC-108 is the homolog of Rab2 in *Caenorhabditis elegans*, which has been shown to regulate apoptotic cell degradation via phagosome maturation in *C. elegans*.³⁷⁻³⁹ *Brucella* recruits Rab2 into their vacuoles via the specific effector RicA and requires Rab2 for replication.⁴⁰⁻⁴² However, this association may be part of the strategy of *Brucella* to interact with the ER, in analogy to the way *Legionella* utilizes Rab1, since no clear role is known for Rab2 in the phagocytic pathway. The presence of Rab2 on phagosomes could nonetheless highlight the importance of interactions between phagosomes and the early secretory pathway or the ER-Golgi intermediate compartment (ERGIC).³²

Rab5

Fusion of phagosomes with endosomes is critical for the process of phagosome maturation.⁴³ Thus, it is not surprising that the majority of the proteomic studies identified isoforms of the early endosomal GTPase Rab5 as being associated with phagosomes.^{8,11-14} In vitro studies using isolated latex bead phagosomes indicated that Rab5 association with phagosomes is lost during

maturation.^{44,45} Purified latex bead phagosomes fuse with early and late endosomal compartments in vitro in a Rab5-dependent manner.⁴⁶ Rab5 is, together with Rab7, one of the best-characterized Rab proteins not only in the endocytic pathway but also in the context of phagosome maturation (see below). Rab5 is required for phagosome maturation and fusion of phagosomes with early endosomes.^{47,48} Most of the initial studies were performed using the expression of the dominant negative mutant to evaluate Rab5 loss-of-function. Subsequently, it was confirmed by knocking down Rab5a, that *Listeria*-containing phagosomes had reduced fusion of phagosomes with lysosomes.⁴⁹ Once associated with phagosomes, Rab5 recruits Early Endosomal Antigen-1 (EEA-1). This, together with phosphatidylinositol 3-phosphate (PI3P) generation at the phagosomal membrane, is critical for maturation of latex bead-containing phagosomes.⁵⁰

Rab7

All the phagosome proteomes considered here have found Rab7 as a phagosomal Rab protein.^{7,8,11-14} Rab7 is required for phagosome maturation in *Dictyostelium*.⁵¹ In mammalian cells the recruitment and activation of Rab7 alone is insufficient to induce fusion of phagosomes with late endosomes and lysosomes.⁴⁷ From

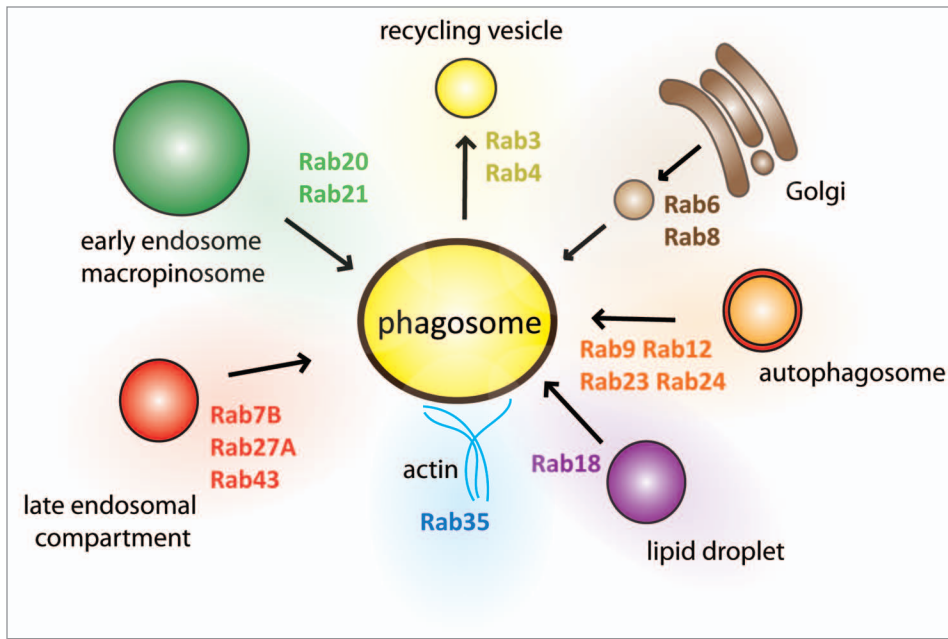


Figure 2. The network of phagosomal Rab GTPases: the less common Rab proteins. This model shows the potential functional link between Rab proteins associated with phagosomes and the putative interactions with different intracellular compartments and/or cytoskeletal components.

the phagosomal membrane, active Rab7 recruits the effector protein Rab7-interacting lysosomal protein (RILP), which in turn brings the microtubule-associated motor complex dynein-dynactin onto phagosomes. These motors not only drive the phagosomes in the centripetal direction but also induce the extension of phagosomal tubules that contact late endocytic compartments.⁵²

Mirroring the mechanism of Rab protein conversion postulated for the endocytic pathway,⁵³⁻⁵⁵ evidence suggests that a similar machinery operates in phagosomes.^{47,56,57} Moreover, it has been proposed that another endocytic Rab GTPase, Rab22a (see below), regulates the conversion from Rab5 to Rab7 in mycobacterial phagosomes.⁵⁸ However, it is not clear which factors Rab22a recruits into phagosomes that could eventually modulate phagosome conversion. On the other hand, it is not entirely clear if this Rab5 to Rab7 conversion observed in the endocytic pathway also applies to the phagocytic pathway since there are several reports suggesting that Rab7 is present in early phagosomes positive for Rab5.^{17,59-61} These data make a compelling case for Rab7 as one of the master regulators of phagosome biology, in particular mediating interactions with the late endocytic/lysosomal compartment.

Rab10

Rab10 has been consistently identified by proteomic studies as a Rab GTPase associated with phagosomes.^{8,11-14} Rab10 is required for endocytosis, recycling, and exocytosis in polarized cells.^{62,63} On one hand, Rab10 was found to be recruited early into IgG-coated latex beads-containing phagosomes where it regulates LAMP-2 acquisition by phagosomes.⁶⁴ On the other hand, other groups have reported a weak association of Rab10 to phagosomes containing *Staphylococcus aureus*,⁶¹ *Mycobacterium tuberculosis*,⁶¹ or *Salmonella*.⁶⁵ These observed differences could be due to the different activation pathways and survival strategies of these

pathogens. In terms of function, it has been proposed that Rab10 could be operating via recycling of components required for phagosome maturation.⁶⁴ It is known that phagosome maturation requires the retrieval of certain phagosomal membrane compounds, a process that also involves the GTPase Rab11.⁶⁶ However, the exact nature of these recycled components mediated either by Rab11 (see below) or Rab10 remain to be identified. Based on recent evidence, Rab10 could also be a potential link between the dynamic interactions between phagosomes and the ER.⁶⁷

Rab11

Isoforms of Rab11 are always present in several particle-containing phagosome proteomes.^{7,8,11,13,14} Originally, it had been shown that Rab11 participates in the mobilization and recruitment of early endocytic compartments in macrophages to enhance phagocytosis.⁶⁸ Expressed

Rab11 is also present in *Salmonella* containing vacuoles.^{65,69} Evidence suggests that Rab11 is part of the essential machinery that regulates phagosome maturation by increasing the recycling of phagosomal components,⁷⁰ likely via the Rab11/Rab4 effector Rab-coupling protein (RCP).⁶⁶ In fact, in vitro studies have shown that there is a recycling mechanism from the phagosome.⁷¹ Although intuitively it would be assumed that during multiple fusions with phagosomes, some membrane gets recycled back to maintain a constant compartment size, the precise mechanism and the nature of the recycled components that are required for phagosome maturation are still an incomplete picture. Interestingly, the immune function of Rab11 in macrophages operates via the regulation of fusion between phagosomes and recycling endosomes. Rab11 regulates the delivery of the Toll-like receptor 4 (TLR4) from endocytic recycling compartments into phagosomes. This recruitment is critical for the intracellular signaling of TLR4 from phagosomes containing Gram-negative bacteria. Moreover, Rab11 is required for TRIF-related adaptor molecule (TRAM) recruitment into phagosomes and interferon regulatory transcription factor 3 (IRF3) signaling leading to the secretion of type I interferons.⁷²

Rab14

This GTPase involved in Trans-Golgi Network (TGN) to early endosomes and plasma membrane transport⁷³ is present in most of the phagosome proteomes.^{7,8,11-14} The first functional evidence of Rab14 in phagosomes came from studies performed in *D. discoideum*. RabD, a *Dictyostelium* Rab14-related GTPase, localizes in the endo-lysosomal pathway and is an important regulator of homotypic phagosome and endo-lysosome fusion.⁷⁴ In macrophages infected with *Mycobacterium bovis* BCG, Rab14 is actively recruited into phagosomes containing mycobacteria,

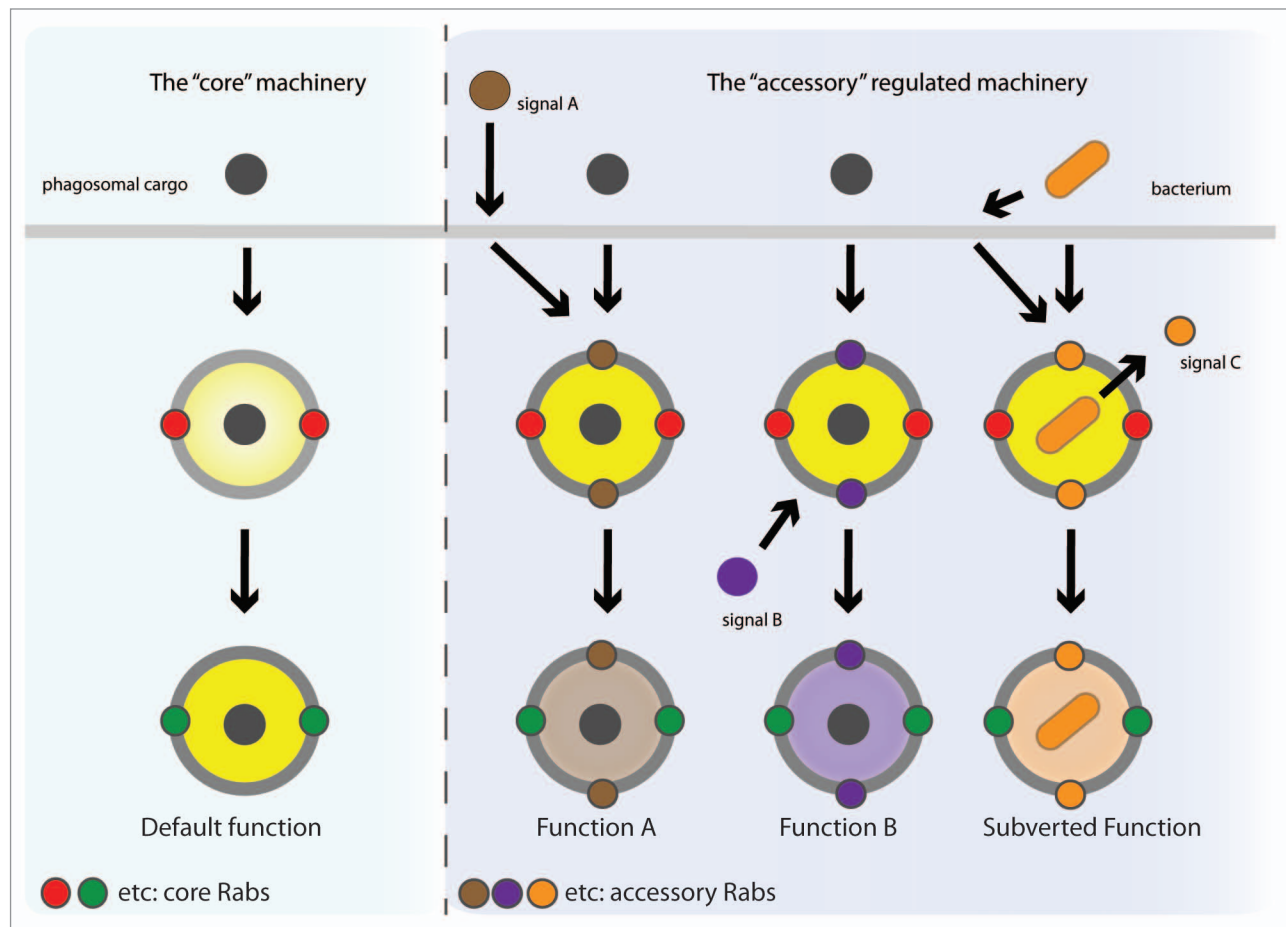


Figure 3. The core vs. accessory network of phagosomal Rab GTPases. Proposed model for the observed functional association of multiple Rab GTPases to phagosomes. A group of 'core' Rab GTPases regulates the default maturation of the phagosomes as housekeeping Rab proteins (indicated in red and green). Another group, called here accessory Rab GTPases could have a functional impact in a more regulated manner (indicated in brown, purple and orange). First, activation via some intracellular (not depicted) and extracellular signals (shown as 'signal A') can change the fate of the phagosome and consequently the function triggered by the origin signal. Alternatively, different intracellular signals e.g., present and/or activated in different cell types (shown as 'signal B') can modulate the default maturation. Finally, compelling evidence indicates that pathogen-driven recruitment (either after activation of receptors or secreting bacterial factors, shown as 'signal C') of Rab GTPases can change the phagosomal fate facilitating bacterial survival. The model is simplified and does not include well-known situations in which the pathogen-subverted Rab functions are in fact from the core machinery.

correlating this association with an impairment in phagosome maturation.⁷⁵ In vitro studies identified Rab14 involvement in fusion between phagosomes and early endosomes, suggesting that Rab14 has a similar function to that of Rab5. In *Salmonella* infected cells, Rab14 is required for intracellular growth of this bacterium.⁷⁶ More information on the functional role of Rab14 in phagosome biology emerged from studies with *S. typhimurium*. The *Salmonella* effector SopB activates Akt1, which in turn phosphorylates AS160, the GTPase activating protein (GAP) of Rab14. This phosphorylation prevents AS160 binding to phagosomal membranes maintaining an active form of Rab14 associated with the phagosome and consequently inhibiting phagosomal maturation.⁷⁶ Altogether, both studies highlight the importance of Rab14 association with *Salmonella* and mycobacteria containing phagosomes to maintain an early phagosomal identity. Interestingly, other pathogens like *Chlamydia* recruit Rab14 and this recruitment is required for enlargement of the Chlamydial replicative niche.⁷⁷ Moreover, in dendritic cells, it has been postulated that

Rab14 participates in the recruitment of the insulin-responsive aminopeptidase (IRAP) into phagosomes, regulating the IRAP-dependent cross-presentation pathway in those cells.⁷⁸

In summary, all these studies point out Rab14 as an important Rab protein that regulates interaction of phagosomes with early endocytic compartments. However, the precise role of Rab14 in phagosomes and how the function is different from Rab5 remains to be identified.

Rab22A

This small GTPase from the endocytic group of GTPases⁷⁹ has been detected in 2 latex bead phagosome proteomes.^{8,14} However, expressed Rab22A in macrophages was not detected in latex bead phagosomes by live cell imaging.⁵⁸ Clearly, the comparison between bulk studies such as Western blot or Mass Spectrometry vs. single-event, dynamic studies such as live cell imaging is not straightforward and represents an important point to be considered.^{58,64} Conversely, Rab22A is associated with *M. bovis* BCG phagosomes during the first 50 min.⁵⁸ Remarkably, in

phagosomes containing *M. bovis* BCG, Rab22A loss-of-function led to the acquisition of Rab7 into these phagosomes, an event that is partially blocked by the bacteria.^{57,80} Thus, it appears that the recruitment of Rab22A into phagosomes is an important step of the Rab5 to Rab7 conversion process in *M. bovis* BCG phagosomes. In contrast to latex beads and BCG-containing phagosomes, more than half of phagosomes containing *M. tuberculosis* H37Rv were positive for expressed Rab22A after 10 min in fixed macrophages.⁶¹ The latter report is in agreement with the localization of Rab22A in early and recycling endosomes⁸¹ since these early compartments interact with *Mycobacterium avium* phagosomes.^{30,48} One important aspect to consider in studies that use Rab22A expression is that the overexpression of Rab22A clearly has an effect on the endocytic pathway.⁸¹ This indicates that the intracellular levels of Rab22A are important and the behavior of the endogenous protein could potentially differ from the overexpressed protein.

Although the molecular components that Rab22A brings into phagosomes to modulate their function remains unknown, it has been described that during infection with *Legionella*, the VipD effector protein prevents the binding of Rab5 and Rab22a to critical downstream effectors such as Rabaptin-5, Rabenosyn-5 and EEA-1 causing a block in lysosomal degradation. Together, this work reveals endosomal trafficking as a host target of *L. pneumophila* and delineates one of the possible underlying molecular mechanisms.⁸²

Rab32

Rab32 was originally reported to regulate mitochondrial dynamics.⁸³ Later, it was shown that Rab32 together with Rab38 regulates melanosome biogenesis and likely other lysosome-related organelles.⁸⁴ Rab32 was found to be associated with latex bead phagosomes after 2 h of internalization.⁸ Seto and coworkers reported that expression of the dominant negative mutant of Rab32 impaired the acquisition of the lysosomal enzyme Cathepsin D by latex bead phagosomes.⁶¹ However, no differences between *M. tuberculosis* H37R virulent (HR7Rv) and H37R avirulent (H37Ra) strains were observed, suggesting that Rab32 recruitment on phagosomes is independent of active mechanisms of bacterial subversion.⁶¹ However, it is not clear if the blockage occurs at the level of immature or mature Cathepsin D, present in early or late endosomes respectively, since immunolocalization does not allow discrimination between both forms. Moreover, these observations are based on the expression of a dominant negative form of Rab32. Knockdown experiments will be important to confirm the function of Rab32 in mycobacterial survival. In a different experimental setting, Smith and coworkers reported low association of expressed Rab32 to wild-type *Salmonella* phagosomes.⁶⁵ However, an effector protein of *Salmonella* targets Rab32 for degradation allowing survival of this pathogen in mammalian cells.⁸⁵ Moreover, silencing of Rab32 increases *Salmonella* survival in macrophages⁸⁵

In summary, Rab32 has been consistently found as a phagosomal Rab protein and must be important during phagosome maturation.^{7,8,12,14} Though evidence suggests a role in interactions between phagosomes and late endosomes, the precise phagosomal function of Rab32 remains to be identified.

Rab34

Rab34 was originally described as being associated with phagosomes in proteomic studies.⁸ Rab34 was also described as being transcriptionally dependent of the transcription factor NF- κ B during the lysosomal-mediated killing of mycobacteria by macrophages.⁸⁶ These observations strongly suggested a role for this GTPase in phagosome maturation. Moreover, it was shown that Rab34 participated in the delivery of Cathepsin D into phagosomes but the mechanism is unclear.⁶¹ Recently, Rab34 was shown to have a critical and specific role in phagolysosome biogenesis operating via a size-dependent mechanism of cargo transfer.⁸⁷ Although Rab34 only transiently interacts with phagosomes, knockdown of endogenous Rab34 or overexpression of the Rab34 dominant negative mutant blocks the fusion of phagosomes with lysosomes. Conversely, expression of Rab34 wild type and the constitutively active mutant enhanced phagolysosome biogenesis independently of Rab7.⁸⁷ These studies support a view in which Rab7 and Rab34 perform a largely distinct, but parallel and maybe even complementary functions during phagosome maturation.⁸⁷

Rab GTPases Less Often Identified in Phagosomes and with Unclear Phagosomal Functions (Fig. 2)

Rab3

ApRab3, a GTPase 78% identical to the human Rab3C, was originally reported to be associated with symbiosomes and accumulates on the maturing phagosomes in the *Aiptasia pulchella* digestive cells.⁸⁸ In mammals, Rab3 has been largely associated with several intracellular mechanisms of exocytosis.⁸⁹ The isoform Rab3C was originally detected in one of the first phagosomal proteomes¹¹ and recently in phagosomes isolated from IFN- γ activated cells.¹⁴ In a lentivirus-based siRNA screening, Rab3B/C was found to be required for antigen cross-presentation in dendritic cells.⁹⁰ Based on these observations, it is proposed that in dendritic cells, internalized bacteria in phagosomes and Rab3B/C-positive recycling endosomes may constitute an exocytic step of cross-presentation.⁹⁰

Rab4

The function of this GTPase in phagosome maturation is not known. It has been proposed that RCP present on phagosomes acts as an intermediate between Rab4 and Rab11, regulating recycling events along the phagocytic pathway.⁶⁶ The porin B (PorB) from *Neisseria* induces the early association of Rab4 to latex bead phagosomes.⁹¹ Expressed Rab4 is present in *Salmonella* containing phagosomes.⁶⁵ Interestingly, the imidazoline-1 receptor (I1R) Nischarin is an effector of both Rab4 and Rab14 and is required for survival and replication of *Salmonella* in host-derived vacuoles.⁹²

Rab6

Rab6, together with Rab33B, coordinate a major intra-Golgi retrograde trafficking pathway but the function of Rab6 in phagosome maturation is not known. This coordination may have parallels with Rab conversion/cascade events that regulate endosomal, phagosomal and exocytic processes.⁹³ Moreover, the

recruitment of 2 Golgi-associated Rab proteins, Rab6 and Rab8, on *Salmonella* containing vacuoles was shown to operate via the effector SipC.⁹⁴

Rab7B

Rab7B has a different function from Rab7^{95,96} and it has also been identified as being associated with phagosomes.^{8,14} The function of Rab7B in the context of phagosome maturation is unknown. However, this association is potentially very interesting since it is expressed in macrophages and associated with late endosomes and lysosomes. After LPS treatment, Rab7B is transported to TLR4-positive endosomes leading to TLR4 degradation and signaling. These findings suggest that Rab7B could be a potential negative regulator of TLR4 signaling from the phagosome by promoting the translocation of TLR4 into lysosomes for degradation.⁹⁷

Rab8

Rab8 function has been linked to diverse processes including cell migration and polarization, neuronal differentiation, and generation of cilia.⁹⁸ Although Rab8 is found in phagosome proteomes, little is known about the function of Rab8 in the context of phagosome maturation. Rab8 has been identified as a component of the *Legionella* containing vacuole from *D. discoideum* suggesting that *Legionella*-containing phagosomes communicate with the secretory pathway.¹⁸ In the case of *Salmonella*, it has been shown that the effector protein SipC specifically binds and recruits host Syntaxin 6 (Stx6) together with other accessory molecules including Rab6 and Rab8 on *Salmonella*-containing vacuoles.⁹⁴

Rab9A, Rab12, Rab23, and Rab24

These Rab proteins have been found in phagosome proteomes of macrophages stimulated with IFN- γ .^{12,14} Interestingly, these small GTPases have all been found to be involved in different steps of autophagy and are present on autophagosomes.⁹⁹⁻¹⁰¹

The expression of the dominant-negative mutant of Rab23 inhibits the fusion of *Salmonella*-containing phagosomes with lysosomes.⁶⁵ Moreover, both Rab23 and Rab9A are regulators of autophagy during Group A *Streptococcus* (GAS) infection. Knockdown of Rab9A or Rab23 impairs the killing of intracellular GAS, suggesting that these GTPases play a role in targeting GAS to autophagy and degradation.¹⁰² Thus, it is likely that these GTPases could represent interesting regulators of interactions between phagosomes and autophagosomes, albeit their role in phagosome maturation remains poorly characterized.

Rab18

In mammals, Rab18 plays a role in controlling the interactions between lipid droplets and the ER,^{103,104} in a process regulated by extracellular signals.¹⁰⁵ Rab18 is involved in lipogenesis as well as in lipolysis, eventually facilitating interaction of lipid droplets with ER membranes and allowing exchange of lipids between these 2 compartments.¹⁰⁶ It has been postulated that the maintenance of Rab18 in *Salmonella* vacuoles contributes to the block in transport of phagosomes to lysosomes.¹⁰⁷ The molecular machinery that regulates lipid body interactions with phagosomes is not well characterized.¹⁰⁸ Based on the evidence discussed here and given the importance of lipid droplets for intracellular pathogens,¹⁰⁹ Rab18 could represent a potential link between lipid

droplets and phagosomes. However, the functional role of this GTPase in phagosome dynamics remains to be identified.

Rab20 and Rab21

These GTPases are localized both in early endocytic compartments. Using a dominant negative expression approach, Rab20 has been found to modulate the acquisition of the acidotropic dye lysotracker by latex bead phagosomes.^{61,110} Expressed Rab20 is associated early to phagosomes.¹¹⁰ Altogether, it is likely that Rab20 has a function in phagosome biology although the mechanism is unknown. In the case of Rab21, this GTPase has been found in phagosomes isolated from IFN- γ treated macrophages.¹⁴ Rab21 function is in the regulation of early endosomal dynamics¹¹¹ and it is associated with macropinosomes in macrophages.¹¹² Thus, it is possible that Rab21 regulates early interactions of phagosomes with early endosomes or macropinosomes.

Rab35

Rab35 regulates actin-dependent phagosome formation by recruiting ACAP2 (ArfGAP with coiled-coil, ankyrin repeats and PH domains 2), which might control actin remodeling and membrane trafficking through ADP-ribosylation factor 6 (Arf6).¹¹³ Rab35 remains associated with early phagosomes after phagosome formation.^{8,13,14} Most of the studies performed to analyze the function of Rab35 indicated that this GTPase regulates assembly of actin filaments during development in *Drosophila*, filopodia formation,^{114,115} and F-actin generation.¹¹⁶ The effect of Rab35 in regulating the localized actin assembly is mediated by the actin-bundling protein fascin.¹¹⁴ Altogether, it could be possible that Rab35 contributes to the machinery that assembles actin in phagosomes, a process known to have profound consequences in the fate of phagosomal cargo.^{117,118}

Conclusions and Outstanding Questions

The functional network of phagosomal Rab GTPases

Based on the phagosome proteomic data from multiple experimental settings, a group of Rab proteins emerged as critical players of phagosome biology. This group, represented in **Figure 1**, highlights some important phagosomal functions of Rab proteins such as interactions with early and late endocytic compartments, phagosomal recycling and communication with the post-Golgi pathway and the ER. With some exceptions, the studies discussed here argue for overlapping functions of phagosomal Rab proteins. In this way, processes like phagosome acidification, acquisition of early/late endosomal markers or delivery of specific lysosomal enzymes appears to be regulated by multiple Rab proteins. However, increasing evidence indicates that single Rab proteins that become associated with the phagosome can have highly specific function(s).

Interactions of phagosomes with early endocytic compartments are crucial for phagosome maturation.⁴³ At least 3 Rab proteins regulate the early interactions of phagosomes with the endocytic pathway. Clearly, Rab5 is at the center of this regulatory network. In addition, another early endocytic Rab, Rab22A, regulates the switch of Rab5 for Rab7. Therefore, Rab22A represents another layer of regulation, perhaps recruiting specific factors that modulate the phagosomal transition from Rab5 to Rab7.

In this context, Rab14 would be a link between early endosome/ phagosome interactions and intracellular signals, e.g., AKT1 activation.⁷⁶

Three GTPases, Rab7, Rab32, and Rab34, are reported to regulate fusion of phagosomes with the heterogeneous late endocytic compartment. However, the precise mechanism by which they act is different. Rab7 regulates primarily the transient interactions whereas Rab34 is required for more complete fusion events.⁸⁷ Additionally, Rab7 links phagosomes with molecular motors, microtubule-mediated movement and tubule formation affecting phagosomal transport and fusion.⁵² In this scenario, Rab32 would contribute to the fusion with a subset of vesicles that mainly contain cathepsin D.⁶¹ Nevertheless, the precise origin and identity of these vesicles positive for both Rab32 and Cathepsin D, remains to be defined.

Regarding the process of phagosomal recycling, Rab11 regulates not only recycling from the phagosomes but also the delivery of TLR4 into phagosomes from recycling endosomes, with important consequences in transcriptional activation and production of cytokines.⁷² In this context, Rab10 could be modulating the recycling of transferrin receptors.⁶⁴

Finally, Rab1 and Rab2 mediate the interactions with the ER, post-Golgi and ERGIC compartments with possible consequences in antigen presentation and phagocytosis. It is important to mention that a pathogen-independent function of these 2 Rab proteins remain to be described. However, they must have a critical role in phagosome maturation since they are both commonly identified as phagosomal Rab proteins.

A core vs. accessory/regulated set of phagosomal Rab GTPases?

Proteomic studies revealed a second group of Rab proteins, some of them less well characterized but potentially contributing to specific immune functions of phagosomes (Fig. 2).

Immune cells respond to extracellular stimuli such as cytokines modifying their intracellular trafficking necessities. Thus, the immune modulated function of Rab proteins represents an important level of regulation to consider.¹¹⁹ In IFN- γ activated macrophages, the network of Rab GTPases associated with phagosomes changes dramatically.^{12,14} Many of the phagosomal Rab proteins are similar to those in non-stimulated cells but additionally new Rab proteins are recruited. This group consists of Rab9A, Rab12, Rab20, Rab21, Rab23, Rab24, and Rab43. Hence, it is likely that these IFN- γ dependent Rab proteins might have an important innate immune function in macrophages.

Another step of regulation is represented by specific functions of different cell types such as macrophages and dendritic cells. More specific immune-related pathways could eventually require different Rab GTPases recruitment into phagosomes. This will eventually lead to further specialized functions such as antigen presentation (via MHC I, MHC II, CD1, etc.) and bacterial degradation. A good example of a cell-specific function is the regulation of cross-presentation in dendritic cells by Rab27A. This GTPase regulates the pH of phagosomes in dendritic cells,¹²⁰ whereas it enhances phagocytosis in macrophages.¹²¹ Intriguingly, Rab27A is not detected in any of the proteomes of

phagosomes or late endocytic organelles performed in dendritic cells or macrophages.¹²²⁻¹²⁴

During internalization of microbial pathogens, there are also pathogen-driven changes in the network of phagosomal Rab GTPases.^{16,61,65,125} One striking example is the highly specific recruitment of Rab29 into *Salmonella enterica* serovar Typhi-containing phagosomes.¹²⁶ Many studies have pointed out that there are not only more but also different Rab proteins in bacteria-containing phagosomes than in particle-containing phagosomes. However, in most of the cases, the functional consequence of this recruitment has not been fully investigated.

Based on the evidence discussed here, it would be possible to reconcile the classical linear model of phagosome maturation (the default pathway) with a more specific e.g., immune regulated pathway (Fig. 3). In this way, during the process of phagosome maturation, a core set of Rab proteins regulates a 'default' transport and maturation of phagosomes. This group could be independent of the ligand, immune signals or cell type and regulates critical functions of the phagosome such as interactions with the early and late endocytic compartments. In addition to the core machinery of phagosomal Rab proteins, further recruited Rab proteins could potentially have a specific function in a temporal, immunological or pathogen-driven context during the life of a phagosome. As seen for other small GTPases during endocytosis,⁹ the dynamic network of phagosomal Rab proteins will potentially reflect their ability to perform a specific immune function (Fig. 3).

The concept of phagosome maturation has a very broad definition and includes biochemical changes in membrane and lumen composition.³ The dynamic network of phagosomal Rab GTPases exposes complex functions that converge in phagosome maturation, organizing which components are delivered into the phagosome and which ones are recycled back. In the last few years, studies started to dissect the specific function of individual phagosomal Rab proteins. Nevertheless, the function of the majority of the phagosomal Rab proteins remains poorly characterized.

Deciphering the functional role of every individual phagosomal Rab and how they interact in complex networks during the process of phagosome maturation is critical to understand the link between phagosome biology and the immune response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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