JLB

Mini-Review

Immune regulation of Rab proteins expression and intracellular transport

Gang Pei, Marc Bronietzki and Maximiliano Gabriel Gutierrez[1]

Research Group Phagosome Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany

RECEIVED FEBRUARY 14, 2012; REVISED MARCH 14, 2012; ACCEPTED MARCH 20, 2012. DOI: 10.1189/jlb.0212076

ABSTRACT

Compartmentalization in cells of the immune system, the focus of this review, facilitates the spatiotemporal organization of cellular responses essential for specialized immune functions. In this process of compartment maintenance, Rab proteins are central regulators of protein-mediated transport and fusion of intracellular structures. It is widely believed that the intracellular concentration of proteins that regulate intracellular transport, including Rab proteins, is constitutively mantained. However, there is a growing body of evidence indicating that transcriptional rates of Rab proteins can be modified. This process is especially evident during immune activation and argues that after activation, these cells require higher levels of Rab proteins. The aim of this review is to discuss evidence showing the increasing links between Rab protein expression and intracellular transport, particularly in monocytes and macrophages. We highlight here biological processes in which the expression of Rab GTPases is selectively regulated, leading to the activation of specific intracellular routes. Further, we focus on the immune regulation of intracellular transport after cytokine activation and microbial infection, with an emphasis in mycobacterial infection. *J. Leukoc. Biol.* 92: 41–50; 2012.

INTRACELLULAR TRANSPORT

All eukaryotic cells have an intricate network of membrane-bound organelles. In these cells, the dynamic flow of membranes and cargo between compartments takes place efficiently, while integrity, structure, and biochemical composition of every intracellular organelle is maintained [1]. Compartmentalization in eukaryotic cells facilitates the organization of cellular reactions, important for specific functions, exerted by different cell types in multicellular organisms. Moreover, membrane trafficking allows not only interaction between intracellular compartments but also communication with the extracellular environment.

The endocytic pathway is responsible for the uptake of components present in the extracellular space and internalization of receptors. In this pathway, the endocytosed material is transported from vesicles (or endocytic vesicles) to the early endosomes, a tubo-vesicular network, where internalized components are sorted for recycling or degradation. In addition, receptor-associated endosomes can activate intracellular signaling pathways—signaling endosomes—in a spatiotemporal manner [2]. Although there are emerging exceptions, endocytosis is classically divided in four general categories: clathrin-mediated endocytosis, caveolae (clathrin-independent), macropinocytosis, and phagocytosis [3, 4]. All of these endocytic mechanisms allow cells to internalize a broad range of extracellular components from single molecules to large particles. The highly dynamic endosomal/lysosomal system of mammalian cells and the trafficking pathways within the endosomal system are fundamental for a wide variety of cellular functions, including immunity. This system allows the constitutive and regulated movement of membrane components between the cell surface and intracellular compartments and it is controlled at the molecular level by several factors [2].

On the other hand, and in parallel to endocytosis, all eukaryotic cells have a system to transport molecules to plasma membrane, and many cells secrete specific proteins into the extracellular environment. The secretion of small signaling molecules, such as hormones or cytokines, is generally performed via exocytosis, which can be constitutive (secretion) or regulated (see below). During exocytosis, molecules are released outside of the cell into the extracellular space. Therefore, exocytosis facilitates cell communication through a variety of signaling molecules, which are released after specific stimuli.

CONSTITUTIVE VERSUS INDUCIBLE INTRACELLULAR TRANSPORT

Intracellular trafficking pathways, which are fundamental to the normal cellular physiology, such as endocytosis, exocytosis, and protein sorting/recycling, are constitutively active in eukaryotic cells. Although most of the essential intracellular

Abbreviations: B-cpx=B cell-complex, BMM=bone marrow macrophage(s), CIITA=MHC class II transactivator, EEA1=early endosome antigen 1, GAP=GTPase activating protein, GDI=GDP dissociation inhibitor, GEF=GTPase exchange factor, HCMV=human CMV, Rabex-5=Rab5 guanine exchange factor, REP=Rab escort protein, RFX=regulatory factor X, SP-A=surfactant protein A, Treg=regulatory T cell

1. Correspondence: Phagosome Biology Group, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany. E-mail: mgg08@helmholtz-hzi.de

transport is, without doubt, constitutively active, certain cell types, in response to specific stimuli, activate selected transport pathways. This is more evident in processes, such as endocytosis of selected proteins induced by specific ligands [4, 5]. Furthermore, specialized cell types display not only signal-activated endocytosis but also other specific transport routes. In some cell types, vesicles that carry proteins along the regulated secretory pathway usually accumulate intracellularly. Subsequently, after the appropriate physiological stimulus, vesicles fuse with the plasma membrane, allowing the secretion of those accumulated proteins [6].

Depending on the cell type, the physiological state, and extracellular signals, specific intracellular transport routes need to be spatiotemporally activated or repressed. This allows not only the coordination but also the integration of those signals into existing pathways. As a result, those signals might increase or decrease rates of specific intracellular membrane trafficking pathways. Although it is assumed that induction of a protein involved in a certain pathway will directly affect this trafficking route, knowledge about how this is regulated just started to emerge recently. For example, a transcriptional control of lysosomal function has been reported by investigating genes sharing a promoter signature that regulates lysosomal functionality [7]. In this study, the increase in the expression of certain lysosomal proteins correlates with the intracellular number of lysosomes, providing evidence for a transcriptional control of lysosomal biogenesis [7]. Therefore, this study highlights the relevance of the transcriptional control of an organelle number, arguing that an increase of the rate of vesicular transport will need higher expression of proteins required for the biogenesis of that organelle. Alternatively, intracellular transport can be the result of high rates of formation, transport, and fusion.

INTRACELLULAR TRANSPORT AND IMMUNITY

In cells of the immune system, intracellular organelle function is crucial and therefore, highly regulated. Intracellular transport and immunity are linked in many ways. One example is the intracellular trafficking and signaling of TLRs within the endocytic pathway [8]. The activation of TLR and its signaling from phagosomes and endosomes is modulated by the environment present in the lumen of those organelles [9–11]. During the immune response, many intracellular trafficking routes are activated in immune cells. In T cells, secretion of ILs and the immune modulation exerted by them is dependent on exocytosis. DCs activate endocytosis for uptake and degradation of antigens following transport to the plasma membrane for presentation and activation of the corresponding T cell subset. Macrophages trigger migration and phagocytosis after infection to eliminate the invading pathogens. Phagocytosis is a specific type of endocytosis in immune-specialized cells, especially in professional phagocytes. This pathway is the main mechanism for uptake of pathogens and cell debris and represents the first line of defense against infection [12, 13].

Additionally, phagocytes, being part of the immune system, posses surface receptors that recognize extracellular immune signals, e.g., ILs or microbial constituents. These receptors induce the appropriate response at the intracellular level. Consequently, phagocytes have to adapt their trafficking needs to enable dynamic and customized reactions to infection. From this point of view, phagocytosis could be considered as an inducible trafficking pathway. Professional phagocytes, after contact with microbes, are recruited at the site of infection, where the phagocytic machinery is activated. Consequently, as a result of a higher rate of phagocytosis induction, more phagosomes are generated and therefore, more degradative compartments are needed to eliminate the pathogenic agents.

Rab PROTEINS AS REGULATORS OF INTRACELLULAR TRANSPORT

One important group of proteins that regulates intracellular transport pathways consists of small, monomeric GTPases. These proteins act as regulators of important aspects of compartment dynamics, such as generation, identity, movement, and function. From this large family of small GTPases, members of the Rab GTPase family regulate critical steps of membrane-bound organelle formation, organizing membrane domains and driving compartment maturation [14]. In humans, there are more than 70 different members, which specifically localize in different intracellular compartments. This complex network of Rab proteins controls the dynamic changes of all intracellular trafficking routes [14–16]. In general, it is accepted that specific Rab proteins are associated to certain intracellular transport pathways. This implies that enhancement of a particular vesicle-mediated transport will require an increase in the levels of those Rab proteins associated to the transport steps.

Rab PROTEINS EXPRESSION: NEWLY SYNTHESIZED VERSUS PRE-EXISTING POOL

Rab GTPases have two states: a GTP-bound, active form and a GDP-bound, inactive form. The cycle of activation and inactivation of Rab proteins plays a critical role in vesicular trafficking between intracellular compartments. The pre-existing cytosolic pool of Rab-GDP in complexes with GDI is targeted to their specific compartments. After transient association of complexes, GDI is released through interaction with GDI displacement factor, and exchange of GDP by GTP is catalyzed by GEF. Rab-GTP is able then to recruit diverse proteins called "Rab effectors", which determine Rab protein functions, controlling diverse processes that include vesicle motility, tethering, and fusion. GTP is then hydrolyzed after interaction with a GAP protein. GDI can remove Rab-GDP from membranes, and Rab-GDP/GDI complexes are recycled and ready for a new round [17].

On the other hand, newly synthesized Rab proteins are recognized and guided by REP, proteins structurally related to GDI, to the geranylgeranyl transferase before targeting them to the membrane delivery cycle [18]. The consensus view is that there are consequently two pools of Rab proteins: one

represented by the newly synthesized bound to REP and a second one that recycles between cytosol and target membranes bound to GDI.

Rab PROTEINS EXPRESSION REGULATED BY CYTOKINES

Evidence for transcriptionally regulated trafficking proteins is mainly provided from studies performed in responses of immune cells to activation and infection, particularly in phagosome maturation (see **Fig. 1**). We therefore focus on this aspect in more detail. As in other intracellular transport routes, members of the Rab protein family are critical regulators of phagosome maturation [19]. Although it is well established that phagosome maturation is selectively modulated by cytokines and microbial components, the precise mechanisms are poorly understood. Cytokines have an impact on phagosome maturation, suggesting a link between intracellular membrane trafficking regulation and immune modulators. How this is achieved at the intracellular level is still poorly defined. We describe here evidence indicating that the expression of Rab proteins is modulated by specific cytokines and associates with a change in the trafficking of phagosomes.

IFN-γ

IFN-γ is a cytokine produced mainly by Th1 cells and NK cells. This IFN, originally called macrophage-activating factor, is the key protective cytokine for macrophages, leading to the induction of diverse antimicrobial activities [20]. Not surprisingly, stimulation of phagosome maturation is one of the mechanisms regulated by IFN-γ.

Priming of macrophages with IFN-γ facilitates phagosomal acidification and subsequent bacterial killing. IFN-γ induces the expression of several antimicrobial proteins, including the NOS, which generates NO and IFN-γ-inducible GTPases [19]. The acidification of *Mycobacterium*-containing phagosomes is increased significantly by IFN-γ treatment. Therefore, IFN-γ has the ability to reverse the limited acidification of phagosomes induced by mycobacteria [21, 22]. Furthermore, IFN-γ is also able to modulate intracellular membrane trafficking to increase phagosome maturation in macrophages via induction of autophagy [23].

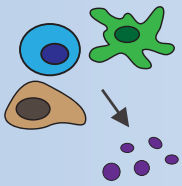
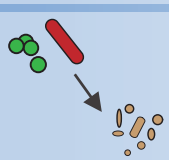
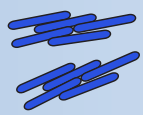
Stimuli	Regulation factors	Rab expression	Correlated immune function	Ref.
 Soluble Mediators	IL-1	↑ Rab3B/C	Ca^{2+}-dependent exocytosis	49
	IL-3	↑ Rab3B/C	Ca^{2+}-dependent exocytosis	49
	IL-4/PGE2	↑ Rab5a	Early endosome dynamics	33
	IL-6	↑ Rab5	Early endosome dynamics	35
		↑ Rab3B/C	Ca^{2+}-dependent exocytosis	49
	IL-12	↑ Rab7	Phagosome/Endosome maturation	35
	IFN-γ	↑ Rab4B	Endosomal recycling (e.g MHC class II)	31
		↑ Rab5a	Early endosome dynamics	24
	SP-A	↑ Rab7b	Late endosome to TGN transport	46
	TGF-β	↑ Rab11	Endosomal recycling	44
	TNF-α	↑ Rab3B/C	Ca^{2+}-dependent exocytosis	47
 Viruses	HCMV	↑ Rab27a	Virus transport, secretion	51
	HIV-1	↑ Rab4A	Endosomal recycling (e.g CD4)	52
 Parasites	*Trypanosoma cruzi*	↓ Rab7	Phagosome/Endosome maturation	53
		↓ Rab11	Endosomal recycling	53
	Theileria annulata	↑ Rab11	Endosomal recycling	54
 Bacterial components	CpG	↓ Rab7b	Late endosome to TGN transport	55
		↑ Rab8A	Lymphocyte activation	56
	Muramyl dipeptide	↓ Rab5	Early endosome dynamics	57
		↑ Rab7	Phagosome/Endosome maturation	57
	LPS	↑ Rab7	Late endosome to TGN transport	58
		↑ Rab10	TLR4 transport from Golgi to plasma membrane	59
		↑ Rab11	Endosomal recycling	60
		↑ Rab37	Exocytosis (e.g TNF-α)	61
 Mycobacteria	*Mycobacterium smegmatis*	↑ Rab10	Transition from nascent to early phagosome	62
		↑ Rab20	Unknown	62
		↑ Rab34	Lysosome positioning, macropinocytosis	62
	Mycobacterium avium	↑ Rab10	Transition from nascent to early phagosome	62
		↑ Rab20	Unknown	62
		↑ Rab34	Lysosome positioning, macropinocytosis	62
	Mycobacterium tuberculosis	↑ Rab8a	Lymphocyte activation, ciliary transport	63
		↑ Rab10	Transition from nascent to early phagosome	63
		↑ Rab20	Unknown	63
		↑ Rab24	Autophagy	64
		↑ Rab32	Autophagy	63
		↓ Rab33A	Putative role in phagolysosome fusion, autophagy	64

Figure 1. Stimuli that affect Rab protein expression and immune-intracellular transport-dependent function. List of the different soluble mediators, viruses, parasites, bacterial molecules, and mycobacteria known to regulate Rab protein expression. The putative, correlated function to a specific Rab protein is also listed in the context of immunity.

One of the first pieces of evidence, showing that immune mediators regulate Rab protein expression, derives from studies performed in macrophages activated with IFN-γ. After IFN-γ treatment, Rab5a transcription was markedly increased in human-derived macrophages [24]. Rab5 is localized to the early endosomes and phagosomes, playing a key role during the maturation of endosomes and phagosomes [25, 26]. Moreover, increased expression of Rab5a correlates directly with accelerated maturation of *Listeria monocytogenes* phagosomes [27].

One of the first events during phagosome maturation is the recruitment of Rab5. Afterwards, type III PI3K is recruited to phagosomes in a Rab5-dependent manner [28, 29]. Phosphatidylinositol-3-phosphate is required for EEA1 protein recruitment to the early phagosomes [30]. As EEA1, Rabenosyn-5, and Rabex-5 form an oligomer in early endosomes, more effector recruitment by Rab5 engages more Rabex-5. In turn, more Rab5 is recruited in a positive-feedback loop, which ensures sufficient Rab5 recruitment to early phagosomes. The extent of Rab5 association to phagosomes can therefore be an effective way to modulate phagosome maturation. Noteworthy, the effect of IFN-γ on Rab5 expression is very specific, as the transcription of Rab5b and Rab5c is not affected. In macrophages, IFN-γ also has an effect on Rab5a function, as the GTP/GDP Rab5a ratio bound to the phagosomal membrane increases twofold in response to IFN-γ. This argues that guanine exchange, GTP hydrolysis, or both are regulated by IFN-γ as well [24].

In DCs, IFN-γ induces the activation of the Rab4B promoter increasing the number of Rab4B transcripts in these APCs. Noteworthy, the promoter of Rab4B contains an enhancer, called S-Y module, regulated by the same transcription factors that regulate MHC class II expression. The S-Y module includes the transcription factor, called RFX, and a transcriptional coactivator known as the CIITA. As Rab4A is associated with early endosomes regulating receptor recycling, the transcriptional activation and coregulation of Rab4B and MHC class II genes argue that antigen presentation is modulated by increasing Rab4B-mediated endosomal recycling of MHC class II [31].

IL-4

The binding of IL-4 promotes alternative activation of macrophages into M2 cells and inhibits classical activation of macrophages into M1 cells [32]. An increase in the number of alternative-activated macrophages (M2) induces high levels of IL-10 and TGF-β, resulting in anti-inflammatory signals. IL-4, in combination with Prostaglandin E2 (PGE2), selectively induces Rab5a expression, supporting the assumption that specific functions are associated with certain Rab5 isoforms [33]. Activation of macrophages with IL-4/PGE2 results in enlarged, early endosomes, a consequence of more fusion or less fission between endosomal vesicles. Indeed, Rab5a is required for the formation of these large endosomes, as Rab5a silencing in IL-4/PGE2-treated macrophages inhibits endosomal enlargement [33]. In this context, it is likely that the higher levels of Rab5a (and perhaps other endosomal proteins) induced by IL-4/PGE2 have an effect in endosome biogenesis. In addition to that IL-4 enhances the proteolitic activity of phagosomes within macrophages [34]. It remains to be determined whether the enhanced proteolysis observed in phagosomes after IL-4 activation is related to the observed alterations in the early endosomal population.

IL-6

The intracellular levels of Rab5 are also higher in IL-6-treated macrophages [35]. IL-6 is a cytokine mainly secreted by T cells and macrophages, which acts as a proinflammatory cytokine, inducing the production of acute-phase proteins in response to infection and injury [36]. In contrast to the effect observed with IFN-γ, all isoforms of Rab5 are induced significantly by IL-6 stimulation. Indeed, the 2kB upstream promoter region of Rab5 is transcriptionally active after IL-6 treatment, indicating that IL-6 specifically triggers a signal transduction pathway, which leads to an increase in the expression of Rab5 [35], and Rab5 expression, activated by IL-6, is regulated by ERKs, as inhibitors of the MEK1/2 signaling pathway abrogate the expression of Rab5 expression induced by IL-6. Consistent with this notion, IL-6 treatment also enhances fusion between early endosomes and early phagosomes in an ERK-dependent manner, in correlation with high Rab5 levels during this process [35]. More important, the effect in Rab5 expression seems to be very specific, as an IL-6-mediated increase in Rab5 promoter activity is blocked specifically by an ERK inhibitor but not by a p38 MAPK inhibitor [35].

IL-12

DCs and phagocytes produce the proinflammatory cytokine IL-12 in response to pathogens during infection, and it is well established that IL-12 is a potent inducer of Th1 responses [37]. The small GTPase Rab7 is specifically up-regulated in macrophages after IL-12 treatment [35]. Rab7 is largely associated to late phagosomes and lysosomes [38, 39]. Its main role is the control and the biogenesis of late endocytic organelles [39]. Furthermore, it is well established that Rab7 is required for phagosome maturation, transport events from late endosomes to lysosomes and autophagy [38, 40, 41]. According to the Rab5-Rab7 transition model, Rab5 is exchanged into Rab7 during phagosome maturation. However, how Rab7 is recruited to phagosome is still poorly defined, although one of the possible components associated to this transition is the homotypic fusion and vacuole protein sorting complex [42, 43]. Interestingly, the 2kB upstream promoter region of Rab7 is transcriptionally active after IL-12 treatment. Although the precise transcription factor(s) involved are not yet identified, this indicates that IL-12 specifically activates intracellular signaling cascades, leading to Rab7 expression. Whereas MEK1/2 regulates Rab5 expression, IL-12 induces Rab7 expression and phagosome maturation in a p38 MAPK-dependent manner. Remarkably, after IL-12 treatment, *Salmonella*-containing phagosomes are significantly more associated to lysosomal markers, indicating that IL-12 may reverse the inhibition of phagosome maturation induced by *Salmonella* [35].

Other immune mediators

Additionally, other immune mediators can modulate Rab expression. Human intestinal epithelial cells display low levels of

Rab11 expression after treatment with TGF-β [44]. Rab11 is one of the master regulators of recycling within the endocytic pathway [45]. TGF-β is important for the regulation of the immune system by Tregs and the differentiation of forkhead box $p3^+$ Tregs and Th17 cells. Although TGF-β appears to block the activation of lymphocytes and phagocytes, the effect of this IL in intracellular transport remains to be elucidated. It can be hypothesized that lower levels of Rab11 will decrease the amount of certain receptors recycled, leading to different responses originated from plasma membrane receptors.

The SP-A modulates the innate immune response in the lung, having a direct effect on alveolar macrophage physiology. SP-A specifically enhances the expression of functionally active Rab7 and Rab7b, but not Rab5 and Rab11, in primary alveolar macrophages [46]. Although SP-A clearly has other effects in macrophage physiology, the increased Rab7 expression stimulated by SP-A correlates with enhanced phagosome maturation [46].

Numerous studies have shown that TNF-α, IL-3, IL-1, and IL-6 promote the differentiation of BMM into osteoclasts. Treatment of BMM with TNF-α, IL-3, IL-1, and IL-6 increases Rab3B/C but not Rab3A expression. The Rab3 subfamily, consisting of four isoforms—Rab3A, -B, -C, and -D—has a different distribution in tissues, indicating specific niche-dependent functions [47, 48]. As Rab3 isoforms are linked to the control of Ca^{2+}-dependent exocytosis, perhaps the expression of specific isoforms of Rab3 is differentially regulated during osteoclastogenesis, leading to exocytosis-mediated release of ILs [49]. In the same way, overexpression of Rab3B in dopaminergic neurons in vivo increases the number and size of synaptic vesicles [50].

Rab EXPRESSION REGULATED BY PARASITES AND VIRUSES

Parasitic infections can also affect Rab expression levels in host cells after infection (**Fig. 1**). The expression of Rab11 and Rab7, but not Rab5, is down-regulated during *T. cruzi* infection in cardiomyocytes [53]. In these studies, alterations in the Rab GTPase expression during *T. cruzi* infection correlate well with an impairment in endocytosis. Despite the fact that the Rab5 protein, levels are normal, the expression of the effector of Rab5 EEA1 (see above) is abnormally low, suggesting that this would be one factor responsible for the decreased endocytosis [53]. *T. annulata*, a parasitic protozoan, which belongs to the phylum *Apicomplexa*, increases the expression of Rab11 in infected B cells. Intriguingly, the up-regulation of Rab11 is very specific, without any effect on other Rab proteins. The majority of Rab11 remains associated with the membrane fraction, arguing that Rab11 is in its GTP-bound, active form. Furthermore, the Rab11 promoter is significantly activated in *Theileria*-infected B cells, arguing that higher expression of Rab11 is a result of transcriptional rather than post-translational activation. Blocking JNK1 signaling reduces AP-1 transactivation of the Rab11 promoter activity [54]. Therefore, the regulation of recycling endosomes and cell-cycle progression might be linked and coordinated during JNK/AP-1 activation in transformed B cells [54].

HCMV increases expression of several Rab genes that control membrane trafficking [65]. Human fibroblast cells infected with HCMV induce a significant increase in Rab27a expression [51], suggesting that high levels of Rab27a could be important for virus transport/secretion and production [51].

The HIV-1 also modulates Rab protein expression during T cell infection. The *tat* gene of HIV-1 stimulates the promoter activity and expression of Rab4A [52]. There are two highly homologous Rab4 isoforms—Rab4A and Rab4B—encoded by two separate genes. Rab4A and Rab4B are localized in the same cellular compartments and have similar functions in recycling. In addition to the regulation of other plasma membrane receptors, Rab4A regulates the surface expression of CD4 via endosome recycling. More important, this increase in CD4 recycling and surface expression mediated by Rab4 controls susceptibility to HIV Infection [52].

Rab EXPRESSION REGULATED BY MICROBIAL COMPONENTS

Activation of TLR9 with DNA-containing CpG motifs inhibits Rab7b expression in macrophages via ERK and p38 MAPK activation [55]. Moreover, in macrophages, Rab7b enhances the transport of TLR9 to the late endosomal/lysosomal compartment, promoting TLR9 degradation. This leads to the suppression of TLR9-triggered production of proinflammatory cytokines, such as TNF-α, IL-6, and IFN-γ [55]. It is known that the expression of B-cpx I is enhanced in response to CpG engagement to TLR9 in activated B cells. Rab8A is a B-cpx I-regulated transcript during the CpG activation in B cells. Although the exact mechanism for Rab8A in lymphocyte function is unknown, both processes are linked to lymphocyte activation, and increased expression of Rab8A could be required during activation-induced proliferation and antigen processing [56].

Muramyl dipeptide is a peptidoglycan constituent of Gram-positive and Gram-negative bacteria. The intracellular delivery of this polymer to macrophages decreases the cellular content of Rab5 and increases the intracellular levels of Rab7. This correlates well with the transport of *Salmonella* to the lysosomal compartment and efficient killing of the bacteria [57]. LPS and IFN-γ treatment also regulates the expression of the recycling endosome protein Rab11 [60]. Activation of TLR4 by LPS enhances Rab10 expression in DCs and macrophages. Moreover, higher Rab10 expression provides a rationale for allowing more TLR4 to traffic to the macrophage modulating TLR4 signaling [59]. The regulation of multiple Rab proteins seems to be organized simultaneously, as whereas LPS induces Rab10 expression, it also enhances the expression of Rab7b in macrophages, leading to more lysosomal degradation of TLR4 [58].

Recently, it has been shown that LPS stimulation significantly induces the expression of Rab37 in primary and RAW 264.7 mouse macrophages. Rab37 is localized to the secretory granules of mast cells, implicating its function in secretory exocytosis of TNF-α [61]. Rab37 silencing using small interfering RNA significantly attenuates the secretion of TNF-α, suggesting that the expression of some Rab proteins and cytokines is stimulated by microbial signals to better fulfill specific immune

functions [66]. All in all, these observations are consistent with the idea that microbial signals can dictate a specific repertoire of Rab protein expression and highlight the relevance of a customized intracellular trafficking response during immune activation.

Rab EXPRESSION REGULATED BY MYCOBACTERIA

In macrophages, the expression of Rab20, Rab34, and Rab10 is up-regulated after infection with *M. smegmatis* and *M. avium* [62]. More important, those small GTPases are regulated at the transcriptional level in a NF-κB-dependent manner. This indicates that during the infection, the mentioned Rab proteins likely play a role during the innate immune response.

Rab20 is localized mainly in early endocytic structures and associated to the Golgi complex [67, 68]. Rab20 partially localizes in compartments positive for the vacuolar proton ATPase in mouse kidney [69]. Given that NF-κB is required for mycobacterial killing, it is tempting to hypothesize that those high levels of Rab20, induced via NF-κB activation, are necessary to cope with the infection. However, the immune function of Rab20 in macrophages or other cells of the immune system are not known.

Rab34 expression induced by mycobacteria also depends on NF-κB activation. Rab34 is localized mainly in the Golgi complex and modulates the positioning of lysosomes [70]. Therefore, Rab34 could potentially modulate lysosomal and/or phagosomal fusion, contributing to phagolysosome biogenesis and bacterial killing. Although Rab34 has been reported to be associated to isolated phagosomes by mass spectrometry [71], the functional contribution of this Rab protein to phagolysosome biogenesis and bacterial killing is not well characterized.

Rab10 regulates trafficking from the Golgi at early stages of cell polarization and is involved in biosynthetic transport to the basolateral membrane [72]. As stated before, LPS enhances Rab10 expression in DCs and macrophages. Moreover, Rab10 expression provides a mechanism to modulate TLR4 signaling by increasing TLR4 surface expression [59]. Further studies have shown that Rab10 is transiently associated with phagosomes at very early stages [73], and it is required for phagosome maturation. The expression of the Rab10 constitutively active mutant increases the percentage of Rab5-positive phagosomes containing *Mycobacterium bovis* bacillus Calmette-Guerin. Therefore, the levels of active (GTP-bound) Rab10 may regulate the transition from nascent phagosomes to early phagosomes [73]. Therefore, it is likely that Rab10 expression via NF-κB is part of a host cell response during early times of mycobacterial infection, which operates to enhance phagosome maturation and killing [62].

In addition to the mentioned cell-based studies, genome-wide transcriptional studies are also contributing enormously to our understanding of Rab expression during *M. tuberculosis* infection. There is an increasing body of evidence from studies performed in patients with tuberculosis that immune cells have different expression levels of Rab proteins, depending on the disease progression [74, 75]. In most of these studies carried out using myeloid cells, the data clearly show that Rab protein expression is differentially regulated during *M. tuberculosis* infection. A comparison of gene expression in PBMCs from tuberculosis versus healthy patients reveals that Rab24 and Rab33A are expressed differently [64]. Rab33A is down-regulated in patients with tuberculosis, and further studies show that Rab33A is preferentially expressed in $CD8^+$ T cells, indicating a putative role of this protein in the adaptive immunity against tuberculosis [64]. Comparative microarray analysis from lung tissues in *M. tuberculosis*-infected versus healthy mice demonstrates that the expression of several Rab genes, such as Rab8a, Rab10, Rab20, Rab24, and Rab32, are up-regulated continuously, suggesting a role in tuberculosis progression [63]. An interesting link emerged from these studies: Rab24, Rab32, and Rab33A have all been implicated in autophagy [76–78]. Curiously, Rab protein expression levels may be up- or down-regulated during infection, only in specific cell types [79]. For example, Rab20 is up-regulated in human macrophages but not in DCs after *M. tuberculosis* infection, arguing for a specific function of this Rab protein in macrophages [79].

All of these genome-wide expression analyses have provided useful information to understand immune-driven responses during infection and inflammation. However, the question of why Rab expression is modified significantly in peripheral blood-derived cells, specifically in patients with active disease, is still elusive. In fact, most of the Rab proteins identified in these studies do not have any obvious, special function in the immune cells. Undoubtedly, this immune cell-specific variation in Rab protein expression is associated to the host response. However, more studies are required to define the functional role of the set of Rab proteins expressed during the immune response to infection.

CONCLUSION AND PERSPECTIVES

Here, we have summarized evidence showing a specific transcriptional regulation of proteins involved in intracellular transport. We focused on how key proteins, such as Rab GTPases, are regulated at the transcriptional level, including specific signaling pathways and transcriptional factors. The molecular mechanisms controlling the rates of Rab protein expression versus recycling/degradation are only beginning to be recognized as important. Most of the evidence, indicating altered gene expression, is derived from studies performed in cells of the immune system. Although our understanding of how Rab expression is regulated during the immune response is still fragmentary, some conclusions about the implicated signaling pathways can be made.

First, several extra- and intracellular signals cooperate in the activation of Rab expression, contributing to the immune response in eukaryotic cells. Specific cytokines, pathogens, or even single bacterial components modulate the intracellular levels of certain Rab proteins. Given that some of these ILs have an effect on intracellular trafficking steps, such as phagocytosis/phagosome maturation or antigen presentation, it is tempting to hypothesize that enhanced expression of different Rab subsets results in a higher rate of activity of the pathway controlled by those Rab proteins. Whether the activity of the

transport is only enhanced or also proceeds faster remains to be determined. In this way, specific immune signals could eventually regulate specific intracellular pathways associated to specific responses (**Fig. 2**).

In agreement with this idea, genome-wide studies in inflammatory responses strength the concept of membrane-trafficking regulation through the control of Rab protein expression. Altered expression of Rab proteins has been found in many diseases where intracellular trafficking is deregulated, such as cardiomyopathy [80], lung, prostate, pancreatic cancer [68, 81, 82], and atherogenesis [83].

The second take-home message is that some of the signaling molecules that mediate Rab expression are shared between several sources of activation. Intriguingly, the intracellular MAPK signaling cascades already associated to Rab expression are also important signaling mediators in some inflammatory responses, exposure to pathogens and stress in general. This highlights the importance of understanding signaling pathways affecting Rab expression during infection and inflammation (Fig. 2).

Finally, specific transcription factors, such as NF-κB and AP-1, have been identified as important for this regulation. Interestingly, both transcriptional activators also regulate gene expression in response to cytokines, stress, and bacterial and viral infection. It is likely that many other transcriptional activators are required for this process, and different combinations will induce different sets of Rab protein expression, conveying multiple signals. These factors remain to be identified (Fig. 2).

All Rab proteins are not expressed in all tissues and cell types, and the Rab protein expression pattern is different between cells. This strongly argues that every cell type produces a specific set of Rab proteins to sustain only certain trafficking steps. Alternatively, the expression of a subgroup of Rab proteins can have different functions in different cell types. However, based on the data discussed here, the intracellular quantity of Rab proteins is also an important rate-limiting factor for vesicular transport regulation. The concept of inducible Rab proteins is exemplified at best during the immune regulation of the endocytic and phagocytic pathways (**Fig. 3**). Evidence from immune activated cells favors the idea that rapid synthesis of new Rab molecules is necessary to cope with specific transport requirements. Of course, Rab proteins are not the only regulators of intracellular transport; other proteins are likewise required, and it is logical to assume that the levels of these proteins also need be regulated for some conditions. Consistent with this notion, Syntaxin 6 and Vti1b are soluble NSF attachment protein receptors, up-regulated in macrophages by LPS, leading to TNF-α secretion [84]. During immune activation of autophagy, different stimuli rapidly increase the membrane flow from compartment generation to fusion and degradation. Are critical components of the autophagic machinery, such as Rab7 expression, also regulated at the transcriptional level? Little is known about the transcriptional regulation of the Rab proteins belonging to the autophagic machinery.

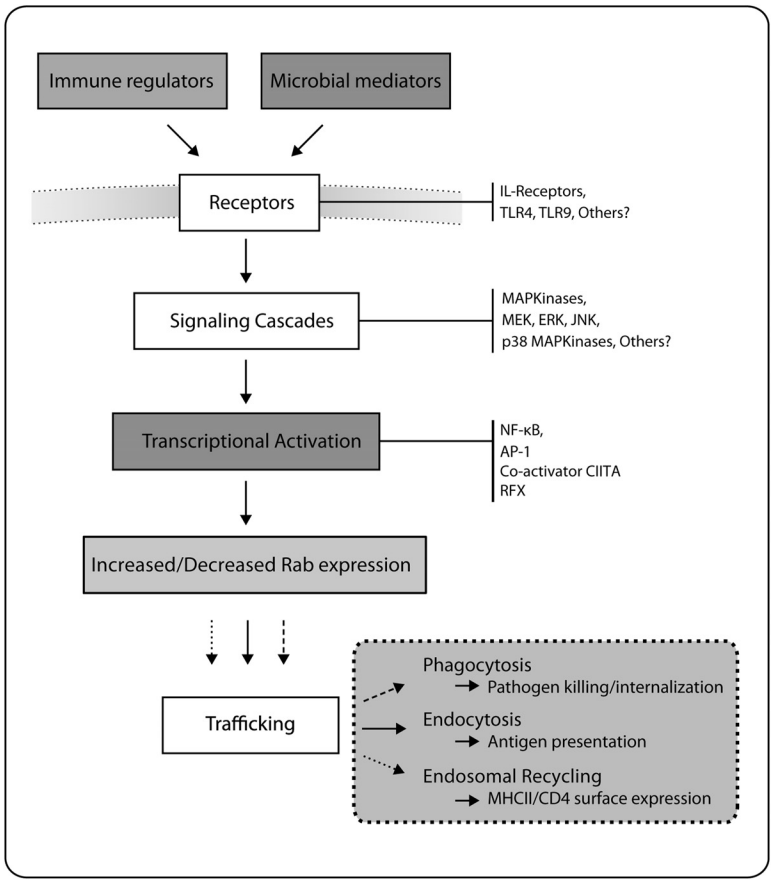


Figure 2. Rab gene expression during immune regulation. Overview of the known components of the pathways linking immune cell activation and trafficking routes. Several immune and microbial modulators activate receptors at the surface (and also from the intracellular compartments). Intracellular signaling pathways are then activated (e.g., several MAPKs). Then, specific transcription factors associate to Rab promoter regions. All of these signals convey in Rab expression and stimulation of selected transport steps triggered by the signals (see text for more details).

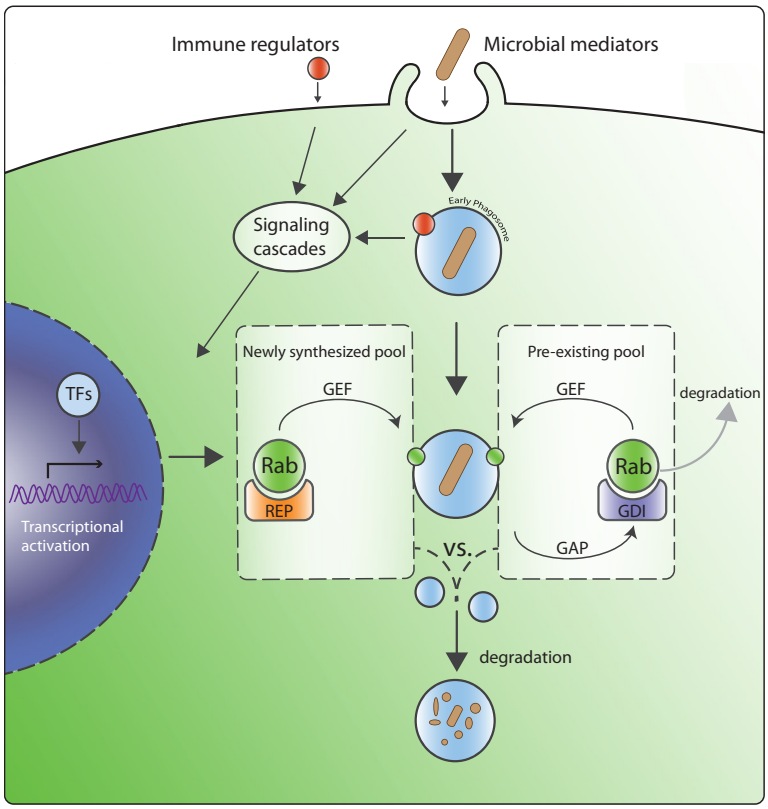


Figure 3. Model showing the link between Rab protein expression and trafficking during phagocytosis. In this model, different signals associated to plasma membrane or intracellular compartments regulate the transcription of Rab proteins through transcription factors (TFs). This increases the amount of newly synthesized proteins in the cytosol. Newly synthesized Rab proteins then interact with REP. Once the proteins are activated to the GTP-bound form by the guanyl nucleotide exchange factor, they associate to the membranes to regulate vesicular trafficking. This membrane-bound pool of proteins can be recycled by a GAP and binds to GDI for further association to membranes and function regulation. This model emphasizes the contrast between the two distinct pools of Rab proteins and raises the question of why in some cases, the newly synthesized pool needs to be increased.

Based on the existing knowledge that we have about Rab protein biology, one important aspect to consider is that the pre-existing pool of Rab proteins can also be recycled. Therefore, one may ask whether it is really necessary to increase the number of Rab molecules that are required for an individual trafficking pathway (Fig. 3). Is the cycling of the cytosolic pool sufficient? Perhaps the two pools have different roles in a temporal context: one fast, which responds to immediate requirements of a certain pathway, and another responsive in the relative long-term to sustain an active transport. To study this in more detail, it is necessary to identify the precise signals that target Rab proteins for degradation. The molecular regulatory proteins that control the internal versus the newly synthesized pool of Rab proteins are evidently different. Clearly, the manipulation by pathogens of the machinery that regulates active versus inactive states of Rab protein activation is a very efficient system to subvert trafficking [85, 86]. But, also, the direct degradation of Rab proteins confers an effective strategy for pathogens to control intracellular transport [87]. In an alternative scenario, Rab protein expression can only be induced after infection in selected immune cells, where the expression is low or totally absent. These specific Rab proteins are good candidates for immune-induced Rab GTPases, with important roles in resistance to infection.

ACKNOWLEDGMENTS

The Phagosome Biology Group and its work in intracellular trafficking are supported by a Helmholtz Young Investigator Grant (Initiative and Networking funds of the Helmholtz Association) and a Priority Program SPP1580 Grant of the German Research Council (Deutsche Forschungsgemeinschaft, DFG). We are grateful to Cristina Vazquez and Gareth Griffiths for critical reading of this manuscript.

REFERENCES

1. Bonifacino, J. S., Glick, B. S. (2004) The mechanisms of vesicle budding and fusion. *Cell* **116,** 153–166.
2. Scita, G., Di Fiore, P. P. (2010) The endocytic matrix. *Nature* **463,** 464–473.
3. Hansen, C. G., Nichols, B. J. (2009) Molecular mechanisms of clathrin-independent endocytosis. *J. Cell Sci.* **122,** 1713–1721.
4. McMahon, H. T., Boucrot, E. (2011) Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell. Biol.* **12,** 517–533.
5. Sorkin, A., von Zastrow, M. (2009) Endocytosis and signalling: intertwining molecular networks. *Nat. Rev. Mol. Cell. Biol.* **10,** 609–622.
6. Stanley, A. C., Lacy, P. (2010) Pathways for cytokine secretion. *Physiology (Bethesda)* **25,** 218–229.
7. Sardiello, M., Palmieri, M., di Ronza, A., Medina, D. L., Valenza, M., Gennarino, V. A., Di Malta, C., Donaudy, F., Embrione, V., Polishchuk, R. S., Banfi, S., Parenti, G., Cattaneo, E., Ballabio, A. (2009) A gene network regulating lysosomal biogenesis and function. *Science* **325,** 473–477.
8. McGettrick, A. F., O'Neill, L. A. (2010) Localisation and trafficking of Toll-like receptors: an important mode of regulation. *Curr. Opin. Immunol.* **22,** 20–27.
9. Underhill, D. M., Gantner, B. (2004) Integration of Toll-like receptor and phagocytic signaling for tailored immunity. *Microbes Infect.* **6,** 1368–1373.
10. Blander, J. M., Medzhitov, R. (2006) On regulation of phagosome maturation and antigen presentation. *Nat. Immunol.* **7,** 1029–1035.
11. Blander, J. M., Medzhitov, R. (2006) Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* **440,** 808–812.
12. Underhill, D. M., Ozinsky, A. (2002) Phagocytosis of microbes: complexity in action. *Annu. Rev. Immunol.* **20,** 825–852.
13. Jutras, I., Desjardins, M. (2005) Phagocytosis: at the crossroads of innate and adaptive immunity. *Annu. Rev. Cell Dev. Biol.* **21,** 511–527.

14. Zerial, M., McBride, H. (2001) Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell. Biol.* **2,** 107–117.
15. Stenmark, H., Olkkonen, V. M. (2001) The Rab GTPase family. *Genome Biol.* **2,** REVIEWS3007.
16. Stenmark, H. (2009) Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell. Biol.* **10,** 513–525.
17. Goody, R. S., Rak, A., Alexandrov, K. (2005) The structural and mechanistic basis for recycling of Rab proteins between membrane compartments. *Cell. Mol. Life. Sci.* **62,** 1657–1670.
18. Shen, F., Seabra, M. C. (1996) Mechanism of digeranylgeranylation of Rab proteins. Formation of a complex between monogeranylgeranyl-Rab and Rab escort protein. *J. Biol. Chem.* **271,** 3692–3698.
19. Flannagan, R. S., Cosio, G., Grinstein, S. (2009) Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nat. Rev. Microbiol.* **7,** 355–366.
20. Schroder, K., Hertzog, P. J., Ravasi, T., Hume, D. A. (2004) Interferon-γ: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.* **75,** 163–189.
21. Via, L. E., Fratti, R. A., McFalone, M., Pagan-Ramos, E., Deretic, D., Deretic, V. (1998) Effects of cytokines on mycobacterial phagosome maturation. *J. Cell Sci.* **111,** 897–905.
22. Schaible, U. E., Sturgill-Koszycki, S., Schlesinger, P. H., Russell, D. G. (1998) Cytokine activation leads to acidification and increases maturation of *Mycobacterium avium*-containing phagosomes in murine macrophages. *J. Immunol.* **160,** 1290–1296.
23. Gutierrez, M. G., Master, S. S., Singh, S. B., Taylor, G. A., Colombo, M. I., Deretic, V. (2004) Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* **119,** 753–766.
24. Alvarez-Dominguez, C., Stahl, P. D. (1998) Interferon-γ selectively induces Rab5a synthesis and processing in mononuclear cells. *J. Biol. Chem.* **273,** 33901–33904.
25. Gorvel, J. P., Chavrier, P., Zerial, M., Gruenberg, J. (1991) rab5 controls early endosome fusion in vitro. *Cell* **64,** 915–925.
26. Stenmark, H., Parton, R. G., Steele-Mortimer, O., Lutcke, A., Gruenberg, J., Zerial, M. (1994) Inhibition of rab5 GTPase activity stimulates membrane fusion in endocytosis. *EMBO J.* **13,** 1287–1296.
27. Alvarez-Dominguez, C., Stahl, P. D. (1999) Increased expression of Rab5a correlates directly with accelerated maturation of *Listeria monocytogenes* phagosomes. *J. Biol. Chem.* **274,** 11459–11462.
28. Schu, P. V., Takegawa, K., Fry, M. J., Stack, J. H., Waterfield, M. D., Emr, S. D. (1993) Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* **260,** 88–91.
29. Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S. C., Waterfield, M. D., Backer, J. M., Zerial, M. (1999) Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nat. Cell Biol.* **1,** 249–252.
30. Burd, C. G., Emr, S. D. (1998) Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. *Mol. Cell* **2,** 157–162.
31. Krawczyk, M., Leimgruber, E., Seguin-Estevez, Q., Dunand-Sauthier, I., Barras, E., Reith, W. (2007) Expression of RAB4B, a protein governing endocytic recycling, is co-regulated with MHC class II genes. *Nucleic Acids Res.* **35,** 595–605.
32. Ishii, M., Wen, H., Corsa, C. A., Liu, T., Coelho, A. L., Allen, R. M., Carson IV, W. F., Cavassani, K. A., Li, X., Lukacs, N. W., Hogaboam, C. M., Dou, Y., Kunkel, S. L. (2009) Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* **114,** 3244–3254.
33. Wainszelbaum, M. J., Proctor, B. M., Pontow, S. E., Stahl, P. D., Barbieri, M. A. (2006) IL4/PGE2 induction of an enlarged early endosomal compartment in mouse macrophages is Rab5-dependent. *Exp. Cell. Res.* **312,** 2238–2251.
34. Balce, D. R., Li, B., Allan, E. R., Rybicka, J. M., Krohn, R. M., Yates, R. M. (2011) Alternative activation of macrophages by IL-4 enhances the proteolytic capacity of their phagosomes through synergistic mechanisms. *Blood* **118,** 4199–4208.
35. Bhattacharya, M., Ojha, N., Solanki, S., Mukhopadhyay, C. K., Madan, R., Patel, N., Krishnamurthy, G., Kumar, S., Basu, S. K., Mukhopadhyay, A. (2006) IL-6 and IL-12 specifically regulate the expression of Rab5 and Rab7 via distinct signaling pathways. *EMBO J.* **25,** 2878–2888.
36. Heinrich, P. C., Castell, J. V., Andus, T. (1990) Interleukin-6 and the acute phase response. *Biochem. J.* **265,** 621–636.
37. Trinchieri, G., Pflanz, S., Kastelein, R. A. (2003) The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* **19,** 641–644.
38. Feng, Y., Press, B., Wandinger-Ness, A. (1995) Rab 7: an important regulator of late endocytic membrane traffic. *J. Cell Biol.* **131,** 1435–1452.
39. Bucci, C., Thomsen, P., Nicoziani, P., McCarthy, J., van Deurs, B. (2000) Rab7: a key to lysosome biogenesis. *Mol. Biol. Cell* **11,** 467–480.
40. Harrison, R. E., Bucci, C., Vieira, O. V., Schroer, T. A., Grinstein, S. (2003) Phagosomes fuse with late endosomes and/or lysosomes by extension of membrane protrusions along microtubules: role of Rab7 and RILP. *Mol. Cell. Biol.* **23,** 6494–6506.
41. Gutierrez, M. G., Munafo, D. B., Beron, W., Colombo, M. I. (2004) Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J. Cell Sci.* **117,** 2687–2697.
42. Rink, J., Ghigo, E., Kalaidzidis, Y., Zerial, M. (2005) Rab conversion as a mechanism of progression from early to late endosomes. *Cell* **122,** 735–749.
43. Poteryaev, D., Datta, S., Ackema, K., Zerial, M., Spang, A. (2010) Identification of the switch in early-to-late endosome transition. *Cell* **141,** 497–508.
44. Lindfors, K., Halttunen, T., Kainulainen, H., Maki, M. (2001) Differentially expressed CC3/TIP30 and Rab11 along in vivo and in vitro intestinal epithelial cell crypt-villus axis. *Life Sci.* **69,** 1363–1372.
45. Ullrich, O., Reinsch, S., Urbe, S., Zerial, M., Parton, R. G. (1996) Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135,** 913–924.
46. Sender, V., Moulakakis, C., Stamme, C. (2011) Pulmonary surfactant protein A enhances endolysosomal trafficking in alveolar macrophages through regulation of Rab7. *J. Immunol.* **186,** 2397–2411.
47. Oishi, H., Sasaki, T., Nagano, F., Ikeda, W., Ohya, T., Wada, M., Ide, N., Nakanishi, H., Takai, Y. (1998) Localization of the Rab3 small G protein regulators in nerve terminals and their involvement in Ca2+-dependent exocytosis. *J. Biol. Chem.* **273,** 34580–34585.
48. Schluter, O. M., Khvotchev, M., Jahn, R., Sudhof, T. C. (2002) Localization versus function of Rab3 proteins. Evidence for a common regulatory role in controlling fusion. *J. Biol. Chem.* **277,** 40919–40929.
49. Abu-Amer, Y., Teitelbaum, S. L., Chappel, J. C., Schlesinger, P., Ross, F. P. (1999) Expression and regulation of RAB3 proteins in osteoclasts and their precursors. *J. Bone Miner. Res.* **14,** 1855–1860.
50. Chung, C. Y., Koprich, J. B., Hallett, P. J., Isacson, O. (2009) Functional enhancement and protection of dopaminergic terminals by RAB3B overexpression. *Proc. Natl. Acad. Sci. USA* **106,** 22474–22479.
51. Fraile-Ramos, A., Cepeda, V., Elstak, E., van der Sluijs, P. (2010) Rab27a is required for human cytomegalovirus assembly. *PLoS ONE* **5,** e15318.
52. Nagy, G., Ward, J., Mosser, D. D., Koncz, A., Gergely Jr., P., Stancato, C., Qian, Y., Fernandez, D., Niland, B., Grossman, C. E., Telarico, T., Banki, K., Perl, A. (2006) Regulation of CD4 expression via recycling by HRES-1/RAB4 controls susceptibility to HIV infection. *J. Biol. Chem.* **281,** 34574–34591.
53. Batista, D. G., Silva, C. F., Mota, R. A., Costa, L. C., Meirelles, M. N., Meuser-Batista, M., Soeiro, M. N. (2006) *Trypanosoma cruzi* modulates the expression of Rabs and alters the endocytosis in mouse cardiomyocytes in vitro. *J. Histochem. Cytochem.* **54,** 605–614.
54. Lizundia, R., Chaussepied, M., Naissant, B., Masse, G. X., Quevillon, E., Michel, F., Monier, S., Weitzman, J. B., Langsley, G. (2007) The JNK/AP-1 pathway upregulates expression of the recycling endosome rab11a gene in B cells transformed by *Theileria*. *Cell Microbiol.* **9,** 1936–1945.
55. Yao, M., Liu, X., Li, D., Chen, T., Cai, Z., Cao, X. (2009) Late endosome/lysosome-localized Rab7b suppresses TLR9-initiated proinflammatory cytokine and type I IFN production in macrophages. *J. Immunol.* **183,** 1751–1758.
56. Porter, J. F., Vavassori, S., Covey, L. R. (2008) A polypyrimidine tract-binding protein-dependent pathway of mRNA stability initiates with CpG activation of primary B cells. *J. Immunol.* **181,** 3336–3345.
57. Mukherjee, K., Parashuraman, S., Krishnamurthy, G., Majumdar, J., Yadav, A., Kumar, R., Basu, S. K., Mukhopadhyay, A. (2002) Diverting intracellular trafficking of *Salmonella* to the lysosome through activation of the late endocytic Rab7 by intracellular delivery of muramyl dipeptide. *J. Cell Sci.* **115,** 3693–3701.
58. Wang, Y., Chen, T., Han, C., He, D., Liu, H., An, H., Cai, Z., Cao, X. (2007) Lysosome-associated small Rab GTPase Rab7b negatively regulates TLR4 signaling in macrophages by promoting lysosomal degradation of TLR4. *Blood* **110,** 962–971.
59. Wang, D., Lou, J., Ouyang, C., Chen, W., Liu, Y., Liu, X., Cao, X., Wang, J., Lu, L. (2010) Ras-related protein Rab10 facilitates TLR4 signaling by promoting replenishment of TLR4 onto the plasma membrane. *Proc. Natl. Acad. Sci. USA* **107,** 13806–13811.
60. Murray, R. Z., Kay, J. G., Sangermani, D. G., Stow, J. L. (2005) A role for the phagosome in cytokine secretion. *Science* **310,** 1492–1495.
61. Masuda, E. S., Luo, Y., Young, C., Shen, M., Rossi, A. B., Huang, B. C., Yu, S., Bennett, M. K., Payan, D. G., Scheller, R. H. (2000) Rab37 is a novel mast cell specific GTPase localized to secretory granules. *FEBS Lett.* **470,** 61–64.
62. Gutierrez, M. G., Mishra, B. B., Jordao, L., Elliott, E., Anes, E., Griffiths, G. (2008) NF-κ B activation controls phagolysosome fusion-mediated killing of mycobacteria by macrophages. *J. Immunol.* **181,** 2651–2663.
63. Gonzalez-Juarrero, M., Kingry, L. C., Ordway, D. J., Henao-Tamayo, M., Harton, M., Basaraba, R. J., Hanneman, W. H., Orme, I. M., Slayden, R. A. (2009) Immune response to *Mycobacterium tuberculosis* and identification of molecular markers of disease. *Am. J. Respir. Cell Mol. Biol.* **40,** 398–409.
64. Jacobsen, M., Repsilber, D., Gutschmidt, A., Neher, A., Feldmann, K., Mollenkopf, H. J., Ziegler, A., Kaufmann, S. H. (2005) Ras-associated small GTPase 33A, a novel T cell factor, is down-regulated in patients with tuberculosis. *J. Infect. Dis.* **192,** 1211–1218.
65. Hertel, L., Mocarski, E. S. (2004) Global analysis of host cell gene expression late during cytomegalovirus infection reveals extensive dysregulation of cell cycle gene expression and induction of *Pseudomitosis* independent of US28 function. *J. Virol.* **78,** 11988–12011.

66. Mori, R., Ikematsu, K., Kitaguchi, T., Kim, S. E., Okamoto, M., Chiba, T., Miyawaki, A., Shimokawa, I., Tsuboi, T. (2011) Release of TNF-α from macrophages is mediated by small GTPase Rab37. *Eur. J. Immunol.* **41,** 3230–3239.
67. Lutcke, A., Parton, R. G., Murphy, C., Olkkonen, V. M., Dupree, P., Valencia, A., Simons, K., Zerial, M. (1994) Cloning and subcellular localization of novel Rab proteins reveals polarized and cell type-specific expression. *J. Cell Sci.* **107,** 3437–3448.
68. Amillet, J. M., Ferbus, D., Real, F. X., Antony, C., Muleris, M., Gress, T. M., Goubin, G. (2006) Characterization of human Rab20 overexpressed in exocrine pancreatic carcinoma. *Hum. Pathol.* **37,** 256–263.
69. Curtis, L. M., Gluck, S. (2005) Distribution of Rab GTPases in mouse kidney and comparison with vacuolar H+-ATPase. *Nephron Physiol.* **100,** 31–42.
70. Wang, T., Hong, W. (2002) Interorganellar regulation of lysosome positioning by the Golgi apparatus through Rab34 interaction with Rab-interacting lysosomal protein. *Mol. Biol. Cell* **13,** 4317–4332.
71. Rogers, L. D., Foster, L. J. (2007) The dynamic phagosomal proteome and the contribution of the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* **104,** 18520–18525.
72. Schuck, S., Gerl, M. J., Ang, A., Manninen, A., Keller, P., Mellman, I., Simons, K. (2007) Rab10 is involved in basolateral transport in polarized Madin-Darby canine kidney cells. *Traffic* **8,** 47–60.
73. Cardoso, C. M., Jordao, L., Vieira, O. V. (2010) Rab10 regulates phagosome maturation and its overexpression rescues *Mycobacterium*-containing phagosomes maturation. *Traffic* **11,** 221–235.
74. Berry, M. P., Graham, C. M., McNab, F. W., Xu, Z., Bloch, S. A., Oni, T., Wilkinson, K. A., Banchereau, R., Skinner, J., Wilkinson, R. J., Quinn, C., Blankenship, D., Dhawan, R., Cush, J. J., Mejias, A., Ramilo, O., Kon, O. M., Pascual, V., Banchereau, J., Chaussabel, D., O'Garra, A. (2010) An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* **466,** 973–977.
75. Maertzdorf, J., Ota, M., Repsilber, D., Mollenkopf, H. J., Weiner, J., Hill, P. C., Kaufmann, S. H. (2011) Functional correlations of pathogenesis-driven gene expression signatures in tuberculosis. *PLoS ONE* **6,** e26938.
76. Munafo, D. B., Colombo, M. I. (2002) Induction of autophagy causes dramatic changes in the subcellular distribution of GFP-Rab24. *Traffic* **3,** 472–482.
77. Hirota, Y., Tanaka, Y. (2009) A small GTPase, human Rab32, is required for the formation of autophagic vacuoles under basal conditions. *Cell. Mol. Life. Sci.* **66,** 2913–2932.
78. Itoh, T., Fujita, N., Kanno, E., Yamamoto, A., Yoshimori, T., Fukuda, M. (2008) Golgi-resident small GTPase Rab33B interacts with Atg16L and modulates autophagosome formation. *Mol. Biol. Cell* **19,** 2916–2925.
79. Tailleux, L., Waddell, S. J., Pelizzola, M., Mortellaro, A., Withers, M., Tanne, A., Castagnoli, P. R., Gicquel, B., Stoker, N. G., Butcher, P. D., Foti, M., Neyrolles, O. (2008) Probing host pathogen cross-talk by transcriptional profiling of both *Mycobacterium tuberculosis* and infected human dendritic cells and macrophages. *PLoS ONE* **3,** e1403.
80. Wu, G., Yussman, M. G., Barrett, T. J., Hahn, H. S., Osinska, H., Hilliard, G. M., Wang, X., Toyokawa, T., Yatani, A., Lynch, R. A., Robbins, J., Dorn II, G. W. (2001) Increased myocardial Rab GTPase expression: a consequence and cause of cardiomyopathy. *Circ. Res.* **89,** 1130–1137.
81. Yao, R., Wang, Y., Lubet, R. A., You, M. (2002) Differentially expressed genes associated with mouse lung tumor progression. *Oncogene* **21,** 5814–5821.
82. Calvo, A., Xiao, N., Kang, J., Best, C. J., Leiva, I., Emmert-Buck, M. R., Jorcyk, C., Green, J. E. (2002) Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. *Cancer Res.* **62,** 5325–5335.
83. Kim, J. Y., Jang, M. K., Lee, S. S., Choi, M. S., Bok, S. H., Oh, G. T., Park, Y. B. (2002) Rab7 gene is up-regulated by cholesterol-rich diet in the liver and artery. *Biochem. Biophys. Res. Commun.* **293,** 375–382.
84. Murray, R. Z., Wylie, F. G., Khromykh, T., Hume, D. A., Stow, J. L. (2005) Syntaxin 6 and Vti1b form a novel SNARE complex, which is up-regulated in activated macrophages to facilitate exocytosis of tumor necrosis factor-α. *J. Biol. Chem.* **280,** 10478–10483.
85. Ingmundson, A., Delprato, A., Lambright, D. G., Roy, C. R. (2007) *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* **450,** 365–369.
86. Murata, T., Delprato, A., Ingmundson, A., Toomre, D. K., Lambright, D. G., Roy, C. R. (2006) The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat. Cell Biol.* **8,** 971–977.
87. Spano, S., Liu, X., Galan, J. E. (2011) Proteolytic targeting of Rab29 by an effector protein distinguishes the intracellular compartments of human-adapted and broad-host Salmonella. *Proc. Natl. Acad. Sci. USA* **108,** 18418–18423.

KEY WORDS:
macrophages · phagosomes · mycobacteria · interleukins