Small GTPases 4:4, 252–254; October/November/December 2013; © 2013 Landes Bioscience

A novel anti-microbial function for a familiar Rab GTPase

Stefania Spanò*,† and Jorge E Galán*

Department of Microbial Pathogenesis; Yale University School of Medicine; New Haven, CT USA

†Current affiliation: School of Medical Sciences; University of Aberdeen; Aberdeen, Scotland, UK

Salmonella enterica **is a bacterial pathogen that closely interacts with its host and replicates intracellularly. It has evolved the ability to create an intracellular membrane vacuole where it can survive and replicate. The nature of the *Salmonella* vacuole is still poorly understood, and although it has some features in common with lysosomes, it serves as a suitable niche for its survival. In contrast to broad-host *Salmonella enterica* serovars, *Salmonella enterica* serovar Typhi (*S.* Typhi) is a host-adapted pathogen that does not have the ability to replicate in any species other than humans. Such host adaptation is manifested at the single cell level since this pathogen is unable to survive in non-human macrophages. We recently reported that a pathway dependent on the Rab GTPase Rab32 and its guanine-nucleotide exchange factor BLOC-3 restricts the growth and survival of *S.* Typhi in non-permissive macrophages. We also found that broad host Salmonellae, such as *S.* Typhimurium, are able to antagonize this pathway by delivering a bacterial effector protein that specifically cleaves Rab32 resulting in its degradation.**

Keywords: Rab GTPases, Rab32, intracellular membrane traffic, *Salmonella*, *Salmonella* Typhi, type III secretion, macrophages, innate immunity, lysosomes, lysosome-related organelles

*Correspondence to: Stefania Spanò; Email: stefania.spano@abdn.ac.uk; Jorge E Galán; Email: jorge.galan@yale.edu

Submitted: 08/26/2013

Revised: 11/15/2013

Accepted: 11/20/2013

Published Online: 12/09/2013

http://dx.doi.org/10.4161/sgtp.27282

Commentary to: Spanò S, Galán JE. A Rab32-dependent pathway contributes to Salmonella typhi host restriction. Science 2012; 338:960–3; PMID:23162001; http://dx.doi.org/10.1126/science.1229224

Intracellular bacterial pathogens have evolved very sophisticated strategies to interact with the endomembrane system of their host cells and ensure that they can create a safe intracellular niche within it. To survive within an intracellular membrane-bound compartment they must be able to acquire limiting nutrients required for growth and avoid degradation by hydrolases of the endo-lysosomal system.[1,2] Rab GTPases are fundamental regulators of intracellular traffic in eukaryotic cells[3] and for this reason they represent a prime target for pathogens to divert intracellular traffic for their benefit. Many bacterial intracellular pathogens have been shown to use different strategies to target Rab GTPases. *Legionella pneumophila*, for example, delivers bacterial effectors that target Rab GTPase by acting as guanine exchange factors (GEFs), GTPase activating proteins (GAP), or as enzymes that introduce post-translational modifications that affect the activation status of specific Rab GTPases.[2,4]

The intracellular bacterial pathogen *Salmonella enterica* is a common cause of food poisoning, transmitted through contaminated water and food. After ingestion it reaches the small intestine where it actively invades the cells of the intestinal epithelium. Once inside the cells, it establishes an intracellular vacuole where it survives and replicates. In order to invade epithelial cells and shape its intracellular niche, *Salmonella enterica* delivers a large number of bacteria effectors through 2 type-III-secretion systems.[5] The strategy used by *Salmonella* to avoid fusion with the lysosomal system is still unclear. The vacuole where *Salmonella* survives and replicates acquires several proteins that are regarded as typical lysosomal markers, including lysosomal-associated membrane protein 1 (LAMP-1), other lysosomal glycoproteins and the vacuolar ATPase.[5,6] Despite having many features in common with lysosomes, the *Salmonella*-containing vacuole is depleted of lysosomal enzymes and constitutes a niche that is suitable for its survival and replication.[6,7]

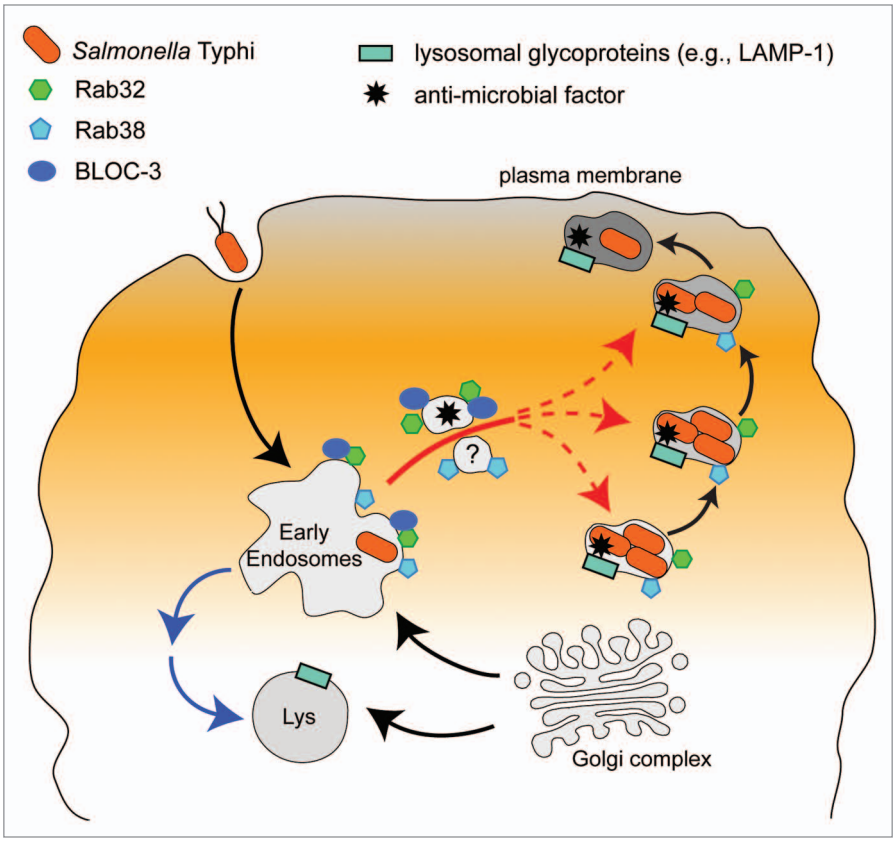

Figure 1. Schematic representation of the maturation of the *S.* Typhi vacuole in mouse macrophages. After internalization, *S.* Typhi (in orange) reaches a compartment that acquires an anti-microbial factor (8-pointed black star) through a transport pathway (red arrow) dependent on the Rab GTPase Rab32 (green hexagon) and its GEF BLOC-3 (blue oval). The *S.* Typhi vacuole also recruits the Rab GTPase Rab38 (cyan pentagon). However, present data suggest that Rab38 is not involved in delivering anti-microbial factors. Like lysosomes and other lysosome-related organelles, the *S.* Typhi vacuole also recruits lysosomal glycoproteins, such as LAMP-1. However, LAMP-1 recruitment to lysosomes (blue arrows) and lysosome-related organelles occurs through distinct pathways.[20,23]

Salmonella enterica can infect a broad range of vertebrate hosts. However, this species comprises more than 2000 different serovars and some of them have adapted to infect a single host. *Salmonella enterica* sv. Typhi (*S.* Typhi) has adapted to infect only humans, where it causes typhoid fever, a life threatening systemic disease that is responsible for more than 200,000 deaths every year.[8,9] Despite its importance as a human pathogen, the molecular bases of its unique pathogenic behavior and human-adaptation are unknown.

Recently we reported that a host transport pathway dependent on Rab32 and the biogenesis of the lysosome-related organelle complex (BLOC)-3 contributes to *S.* Typhi host restriction.[10] We had previously shown that Rab29, a poorly characterized Rab GTPase, is recruited to the *S.* Typhi-containing vacuole, but not to the vacuole of broad-host-range *Salmonella enterica* serovars.[11] This represented the first report of a difference between the intracellular biology of the vacuole of *S.* Typhi and broad-host range *Salmonella enterica* serovars. We found that this difference was due to a novel type-III-secretion effector, GtgE, exclusively expressed by broad-host-range *Salmonella* serovars, which specifically targets Rab29 for cleavage and degradation.[11] *S.* Typhi is not able to survive in primary macrophages derived from mice, a species non-susceptible to *S.* Typhi infection.[12,13] Remarkably, we observed that a *S.* Typhi strain engineered to express GtgE is able to survive in primary macrophages and tissues from mice, a non-permissive species, thus partially overcoming the host-restriction barrier. How does GtgE confer *S.* Typhi the ability to persist in mouse macrophages? We found that, in addition to Rab29, GtgE targets 2 other Rab GTPases, Rab32 and Rab38, which are also recruited to the *S.* Typhi vacuole when expressed in epithelial cells or macrophages.[10] Depletion of Rab32, but not of Rab38 and Rab29, resulted in a marked increase in the survival of *S.* Typhi in mouse macrophages, thus phenocopying the effect of GtgE expression in *S.* Typhi. These results indicate that a Rab32-dependent pathway restricts the growth of *S.* Typhi in mouse macrophages and contributes to *S.* Typhi host restriction.

Rab32 has been shown to have a key role in a post-Golgi transport pathway delivering melanocytic enzymes to maturing melanosomes.[14] The closely related Rab GTPase Rab38 also functions in the same transport pathway and along with Rab32 appears to have both redundant and unique roles in the trafficking of melanin-producing enzymes.[14-16] It is intriguing that Rab38 appears to be dispensable for *S.* Typhi host restriction. It is possible that Rab38 is expressed at low levels in macrophages and that these levels are not sufficient to support any function in these cells. Another possibility is that, while Rab32 delivers an anti-microbial cargo to the *S.* Typhi vacuole, Rab38 would deliver a different type of cargo. These results support the idea that Rab32 and Rab38 play distinct roles in membrane trafficking.

A set of autosomal recessive human disorders, which are grouped under the name of Hermansky-Pudlak Syndrome (HPS) and manifest with albinism and bleeding, are the consequence of defects in the maturation of lysosome-related organelles (LRO), such as melanosomes and platelet dense granules.[17] Human HPS and the corresponding mouse syndrome are associated with mutations in different genes including Rab38, a Rab geranyl-geranyltransferase, the clathrin adaptor protein complex AP-3, Vps33a and components of BLOC-1, -2 and -3. We found that one of the 3 BLOC complexes, BLOC-3, is also required for restriction of *S.* Typhi growth in mouse macrophages.[10] Although the function of the BLOCs in biogenesis of lysosome-related organelles is well established, their specific roles in this process are poorly understood. Interestingly, very

recently BLOC-3 was shown to be a guanine-nucleotide exchange factor (GEF) for Rab32 and Rab38.[18]

Our results indicate that a traffic pathway controlled by Rab32 and its GEF BLOC-3, with features in common with LRO biogenesis, restricts the growth of *S.* Typhi in mouse macrophages (**Fig. 1**). Other observations also indicate that the *S.* Typhi-containing vacuole exhibits features common to LRO. For example, LRO and the *S.* Typhi vacuole contain lysosomal glycoproteins, such as LAMP-1 and LAMP-2, and lack the mannose-6-phosphate receptor, but, in contrast to lysosomes, they contain a specific set of soluble enzymes and transmembrane transporters.[19,20] Based on shared components of the maturation pathway and membrane markers we thus propose that the compartment targeted by *Salmonella* in macrophages be considered a LRO. Intriguingly, intersecting the LRO pathway could represent a strategy for *Salmonella* to avoid to be targeted to lysosomes and degraded in macrophages.

Most Rab GTPases are highly conserved across mammalian species. In contrast, Rab32 shows a significant degree of sequence variation among different mammals, suggesting that it is under selective pressure and that its variation may have been driven by the action of virulence factors, such as the *Salmonella* effector GtgE, targeting this anti-microbial pathway. This observation also implies that other pathogens may target this pathway. Consistent with this hypothesis, a recent genome-wide study reported that Rab32 single nucleotide polymorphisms are associated with increased susceptibility to *Mycobacterium leprae* infections.[21] In addition, Rab32 is recruited onto the vacuole containing *Mycobacterium tuberculosis*.[22] All together these observations strongly suggest that Rab32 can also be involved in a host-defense mechanism acting on *Mycobacterium* species. In conclusion, our findings highlighted a Rab32-dependent anti-microbial mechanism targeting *S.* Typhi and possibly other pathogens in macrophages. They also underscored the critical role of a single bacterial effector in defeating a host-defense mechanism.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Cossart P, Roy CR. Manipulation of host membrane machinery by bacterial pathogens. Curr Opin Cell Biol 2010; 22:547-54; PMID:20542678; http://dx.doi.org/10.1016/j.ceb.2010.05.006
2. Stein MP, Müller MP, Wandinger-Ness A. Bacterial pathogens commandeer Rab GTPases to establish intracellular niches. Traffic 2012; 13:1565-88; PMID:22901006; http://dx.doi.org/10.1111/tra.12000
3. Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. Physiol Rev 2011; 91:119-49; PMID:21248164; http://dx.doi.org/10.1152/physrev.00059.2009
4. Alix E, Mukherjee S, Roy CR. Subversion of membrane transport pathways by vacuolar pathogens. J Cell Biol 2011; 195:943-52; PMID:22123831; http://dx.doi.org/10.1083/jcb.201105019
5. Bakowski MA, Braun V, Brumell JH. Salmonella-containing vacuoles: directing traffic and nesting to grow. Traffic 2008; 9:2022-31; PMID:18778407; http://dx.doi.org/10.1111/j.1600-0854.2008.00827.x
6. Steele-Mortimer O. The Salmonella-containing vacuole: moving with the times. Curr Opin Microbiol 2008; 11:38-45; PMID:18304858; http://dx.doi.org/10.1016/j.mib.2008.01.002
7. Drecktrah D, Knodler LA, Howe D, Steele-Mortimer O. Salmonella trafficking is defined by continuous dynamic interactions with the endolysosomal system. Traffic 2007; 8:212-25; PMID:17233756; http://dx.doi.org/10.1111/j.1600-0854.2006.00529.x
8. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid Fever. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2010; 50:241-6.
9. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med 2002; 347:1770-82; PMID:12456854; http://dx.doi.org/10.1056/NEJMra020201
10. Spanò S, Galán JEA. A Rab32-dependent pathway contributes to Salmonella typhi host restriction. Science 2012; 338:960-3; PMID:23162001; http://dx.doi.org/10.1126/science.1229224
11. Spanò S, Liu X, Galán JE. Proteolytic targeting of Rab29 by an effector protein distinguishes the intracellular compartments of human-adapted and broad-host Salmonella. Proc Natl Acad Sci U S A 2011; 108:18418-23; PMID:22042847; http://dx.doi.org/10.1073/pnas.1111959108
12. Schwan WR, Huang XZ, Hu L, Kopecko DJ. Differential bacterial survival, replication, and apoptosis-inducing ability of Salmonella serovars within human and murine macrophages. Infect Immun 2000; 68:1005-13; PMID:10678900; http://dx.doi.org/10.1128/IAI.68.3.1005-1013.2000
13. Vladoianu IR, Chang HR, Pechère JC. Expression of host resistance to Salmonella typhi and Salmonella typhimurium: bacterial survival within macrophages of murine and human origin. Microb Pathog 1990; 8:83-90; PMID:2190062; http://dx.doi.org/10.1016/0882-4010(90)90072-X
14. Wasmeier C, Romao M, Plowright L, Bennett DC, Raposo G, Seabra MC. Rab38 and Rab32 control post-Golgi trafficking of melanogenic enzymes. J Cell Biol 2006; 175:271-81; PMID:17043139; http://dx.doi.org/10.1083/jcb.200606050
15. Bultema JJ, Di Pietro SM. Cell type-specific Rab32 and Rab38 cooperate with the ubiquitous lysosome biogenesis machinery to synthesize specialized lysosome-related organelles. Small GTPases 2013; 4(1):16-21; PMID:23247405
16. Lopes VS, Wasmeier C, Seabra MC, Futter CE. Melanosome maturation defect in Rab38-deficient retinal pigment epithelium results in instability of immature melanosomes during transient melanogenesis. Mol Biol Cell 2007; 18:3914-27; PMID:17671165; http://dx.doi.org/10.1091/mbc.E07-03-0268
17. Di Pietro SM, Dell'Angelica EC. The cell biology of Hermansky-Pudlak syndrome: recent advances. Traffic 2005; 6:525-33; PMID:15941404; http://dx.doi.org/10.1111/j.1600-0854.2005.00299.x
18. Gerondopoulos A, Langemeyer L, Liang JR, Linford A, Barr FA. BLOC-3 mutated in Hermansky-Pudlak syndrome is a Rab32/38 guanine nucleotide exchange factor. Current biology: CB 2012; 22:2135-9.
19. Dell'Angelica EC, Mullins C, Caplan S, Bonifacino JS. Lysosome-related organelles. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 2000; 14:1265-78.
20. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol 2009; 10:623-35; PMID:19672277; http://dx.doi.org/10.1038/nrm2745
21. Zhang F, Liu H, Chen S, Low H, Sun L, Cui Y, Chu T, Li Y, Fu X, Yu Y, et al. Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy. Nat Genet 2011; 43:1247-51; PMID:22019778; http://dx.doi.org/10.1038/ng.973
22. Seto S, Tsujimura K, Koide Y. Rab GTPases regulating phagosome maturation are differentially recruited to mycobacterial phagosomes. Traffic 2011; 12:407-20; PMID:21255211; http://dx.doi.org/10.1111/j.1600-0854.2011.01165.x
23. Groux-Degroote S, van Dijk SM, Wolthoorn J, Neumann S, Theos AC, De Mazière AM, Klumperman J, van Meer G, Sprong H. Glycolipid-dependent sorting of melanosomal from lysosomal membrane proteins by lumenal determinants. Traffic 2008; 9:951-63; PMID:18373728; http://dx.doi.org/10.1111/j.1600-0854.2008.00740.x