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Current Opinion in Cell Biology

Intracellular niche switching as host subversion strategy of bacterial pathogens

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Abstract

Numerous bacterial pathogens "confine" themselves within host cells with an intracellular localization as main or exclusive niche. Many of them switch dynamically between a membrane-bound or cytosolic lifestyle. This requires either membrane damage and/or repair of the bacterial-containing compartment. Niche switching has profound consequences on how the host cell recognizes the pathogens in time and space for elimination. Moreover, niche switching impacts how bacteria communicate with host cells to obtain nutrients, and it affects the accessibility to antibiotics. Understanding the local environments and cellular phenotypes that lead to niche switching is critical for developing new host-targeted antimicrobial strategies, and has the potential to shed light into fundamental cellular processes.

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Current Opinion in Cell Biology 2022, **76**:102081

This review comes from a themed issue on **Membrane Trafficking**

Edited by **Chiara Zurzolo** and **Benjamin Glick**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2022.102081

Changing concepts of intracellular niche formation

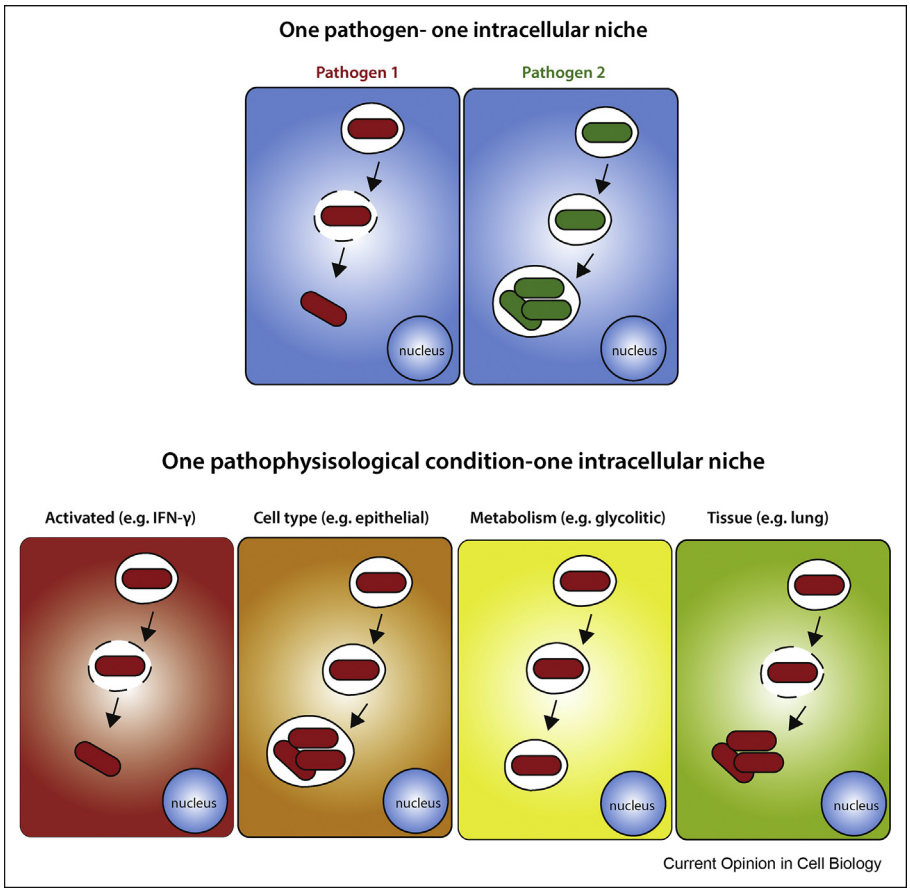
Intracellular bacterial pathogens subvert distinct host pathways to reach their preferred niches within targeted cells [17]. During the last years, our understanding of intracellular bacterial niches has fundamentally changed mainly because of two reasons. First, for a number of bacterial invaders it has been recognized that different intracellular niches (instead of one pathogen-specific one) can be reached during host cell challenge [47]. For example, *Salmonella enterica* or *Mycobacterium tuberculosis* cytosolic access is now recognized as major infection step [7,8]. Secondly, it emerges that the intracellular niche formation depends on an intimate crosstalk between bacterial effectors or toxins that subvert host processes to form a given niche [39]. Escape from a membrane-bound compartment by *Shigella flexneri*, *Listeria monocytogenes* or *M. tuberculosis* is now understood to involve the reprogramming of host pathways [7,48]. The development of novel technologies largely inspired by cell biologists, such as novel biosensors or advanced imaging has become key to tackle these concepts [35]. We are moving away from the dogma "*one pathogen-one intracellular niche*" towards a view that proposes "*one pathophysiological condition-one intracellular niche*" (Figure 1). This can be coined as "*conditioned niche hypothesis*," where the intracellular bacterial localization is appreciated in the context of the infected cell type, tissue, metabolic, and activation status as well as the cellular environment.

Subversion of host trafficking to live within a membrane-bound compartment

The first intracellular bacterial niche is membrane-bound; it is often called phagosome for professional phagocytes or bacterial containing vacuole (BCV) for non-professional phagocytic cells. Formation, maturation and trafficking of phagosomes and BCVs is distinct for each bacterial species as a result of a finely regulated subversion of the host by bacterial effectors (Figure 2). The characterization of the underlying molecular processes has led to a sophisticated understanding of the host subversion strategies by different pathogens, and has further revealed numerous fundamental cell biological concepts for membrane trafficking [39].

Delivery of effectors or toxins across the membrane barrier engulfing the pathogen is a prerequisite for the reprogramming of host pathways. This crosstalk has recently been visualized during *Legionella pneumophila* cell entry combining optical and *in cellulo* structural studies [5]. It revealed discrete interactions between the bacterial type 4 secretion system (T4SS) with the BCV membranes [5]. For another bacterial effector delivery tool, the needle-like *Shigella* type 3 secretion system (T3SS) interactions of the T3SS tip with the

Figure 1



A new concept of intracellular pathogenesis by bacterial pathogens. Changing views on intracellular niche formation. Left: one pathogen (red) via specific virulence factors colonizes one specific niche, whereas another pathogen (green) expressing a different set of factors colonizes a different niche. Right: a new view on intracellular niche formation where the same pathogen (red) localizes in different intracellular niches depending on the specific cellular condition taking into account the activation status, cell type or metabolic and tissue environments. We call this concept "the conditioned niche hypothesis."

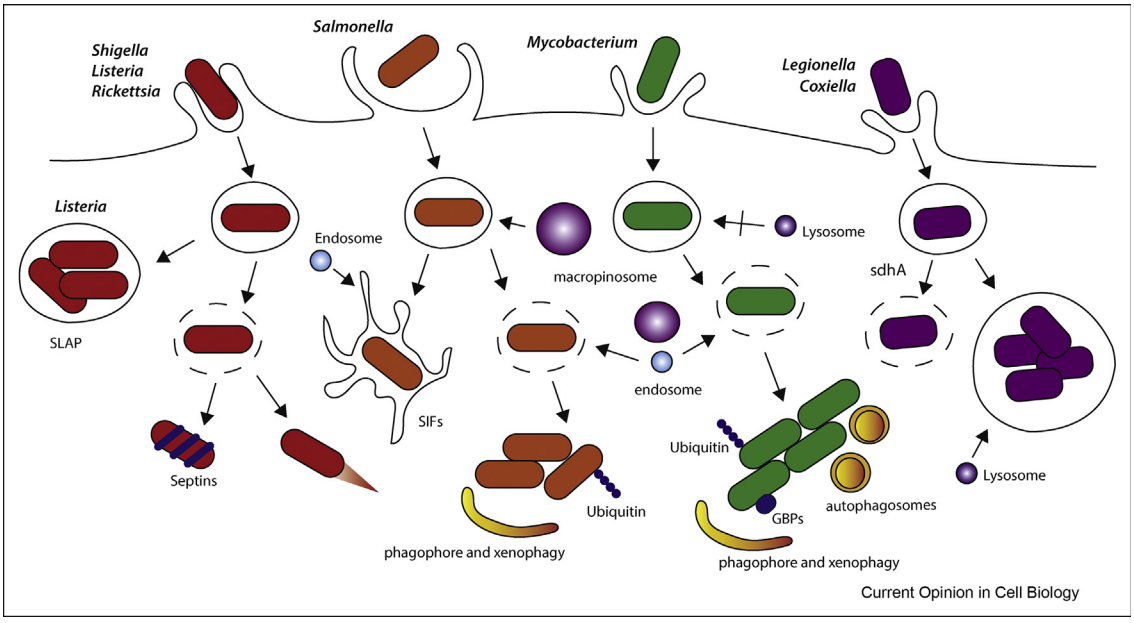
cytoskeleton underneath the BCV membrane are important to regulate the delivery of bacterial proteins into the host cytosol during early BCV formation [53,54].

Recently, BCV formation and interactions with surrounding compartments have been characterized in detail for *S. enterica* invasion of enterocytes, showing that actively entering bacteria are not macropinocytosed, however newly formed macropinosomes interact only at a later stage of pathogen internalization with the BCV to control its size and stability [19,63]. Unexpected links between the BCV and host compartments were also revealed for *Coxiella burnetii*, it was shown that this bacterium needs to engage with lysosomal proteases, such as TPP1 to support its own virulence [42]. *Coxiella* also subverts the Rab GTPase Rab26 to interact with another antimicrobial machinery-the autophagy system-for its own benefit [59].

The vacuolar niche can also be beneficial for typically cytosolic pathogens. In the case of *Listeria* vacuolar bacteria have recently been identified in hepatocytes and trophoblasts where they persist for multiple days [26]. Later, vacuolar growth of *Listeria* was assessed within colon-derived epithelial cells, and unlike in macrophages the virulence factor listerolysin O supports a hyper-replicative phenotype in spacious *Listeria* containing phagosomes in this cell type [46]. Transcytosis of *Listeria* within vacuoles plays an important role in the transport of the pathogen from the apical to the basolateral side of the intestine. The molecular processes taking place during these events could be recently imaged dynamically using light sheet microscopy and organoids [25].

These recent discoveries highlight that the host membrane trafficking machinery is undermined through varied, pathogen-specific compartmental reprogramming

Figure 2



The complexity and dynamics of bacterial pathogen intracellular niches. Schematic representation of recent concepts and niches of bacterial pathogens. The scheme shows the diversity of intracellular niches. The *Salmonella* BCV grows through interactions with endosomes and macropinosomes to form SIFs to acquire nutrients in epithelial cells, or *Salmonella* replicates in the cytosol. Generally, pathogens like *Shigella*, *Listeria* and *Rickettsia* damage the phagosomal membrane and form actin tails. However, in some cell types, *Listeria* also replicates in Spacious *Listeria*-containing vacuoles (SLAPs). Cystosolic *Shigella* are recognized by septins for restriction. Also ubiquitin and xenophagy targeting/tagging of cytosolic bacteria leads to their restriction. Virulent mycobacteria also damage the phagosomal membrane and replicate in the cytosol sometimes forming long cords. These bacilli interact with the autophagy pathway. Depending on the activation status of the host cell, they are subsequently targeted via xenophagy for restriction. *Coxiella* and *Legionella* normally replicate in vacuoles that interact with ER (*Legionella*), or with lysosomes (*Coxiella*). *Legionella* and *Salmonella* lacking specific virulence factors such as sdhA or SopF destabilize the phagosomal membrane. The figure illustrates only some examples of ubiquitination.

strategies for the establishment of the preferred pathogen niche.

Damage and repair mechanisms of bacteria-containing compartments

A radical way to overcome issues related with a lifestyle within a membrane-bound niche, such as dealing with lysosomes or to simplify nutrient acquisition, is the escape into the cytosol through the rupture of the BCV membrane. This strategy has been known for decades for *Listeria*, *Shigella* or *Francisella tularensis* [48]. For them, BCV rupture takes place early upon internalization into host cells and has been associated with their capacity to directly damage host membranes through the action of secreted bacterial pore forming toxins/proteins or dedicated molecular weapons, such as the T3SS. Numerous assays have been developed to measure the step of BCV damage and/or rupture at an ever-increasing spatio-temporal resolution [51]. It is now possible to visualize very early events of lipid flipping at the BCV that precede its disruption with cytosolic sphingomyelin, and likely other lipids, as early markers of membrane damage [14,51]. Nevertheless, it should be noted that in some cases the study of cells with bacteria in damaged compartments can be experimentally challenging as the damage event leads to rapid cell death [32].

BCV damage is often not achieved directly through the action of bacterial factors on the host membranes to destabilize them, instead they interfere with host components that either enhance membrane damage or repair. The number of such host factors is growing, for example, the serine/threonine kinase TAOK2 is hijacked by *Listeria* to damage its intracellular compartment [50]. Also, ROS production and the resulting lipid peroxidation have been shown to promote the rupture of phagosomal membranes [13]. In the case of *Shigella*, a Rab GTPase cascade involving Rab11, Rab8 and the exocyst has been identified for the reprogramming of macropinosomes leading to macropinosome clustering around the BCV [9]. The resulting macropinosomes-BCV contacts are required for efficient disassembly of the damaged BCV membranes [9].

In the case of *M. tuberculosis* infection of macrophages a link between Rab8A, as well as Rab20 and Parkinson's disease (PD)-related kinase LRRK2 has been found to stabilize phagosomal integrity indicating that the

subversion of Rab GTPases is different compared to *Shigella* [22,34,58]. Pathogen-specificity of host subversion is also exemplified for *S. enterica* infection of enterocytes. The cytosolic bacterial pool (as a result of BCV damage) increases during invasion in case BCV-macropinosome contacts cannot occur for BCV growth contrasting macropinosome subversion during *Shigella* invasion [9,63]. Regulation of compartmental growth and shrinkage has also been identified to be important during phagosomal damage during fungal infection [66]. Thus, communication of the damaged bacterial compartment with surrounding host factors and compartments represents a major paradigm for the dynamic balance of compartmental rupture or repair (Figure 3). This is striking for *M. tuberculosis* that switches dynamically between a membrane-bound and cytosolic localization where recapture by xenophagy is critical, similar to a process that has been proposed for the stabilization of *Salmonella* BCVs [4,27]. In addition, the ESCRT-III complex is a central factor supporting membrane repair through the recruitment of novel intact membranes from surrounding compartments, like endosomes or macropinosomes, [34,38,61,63].

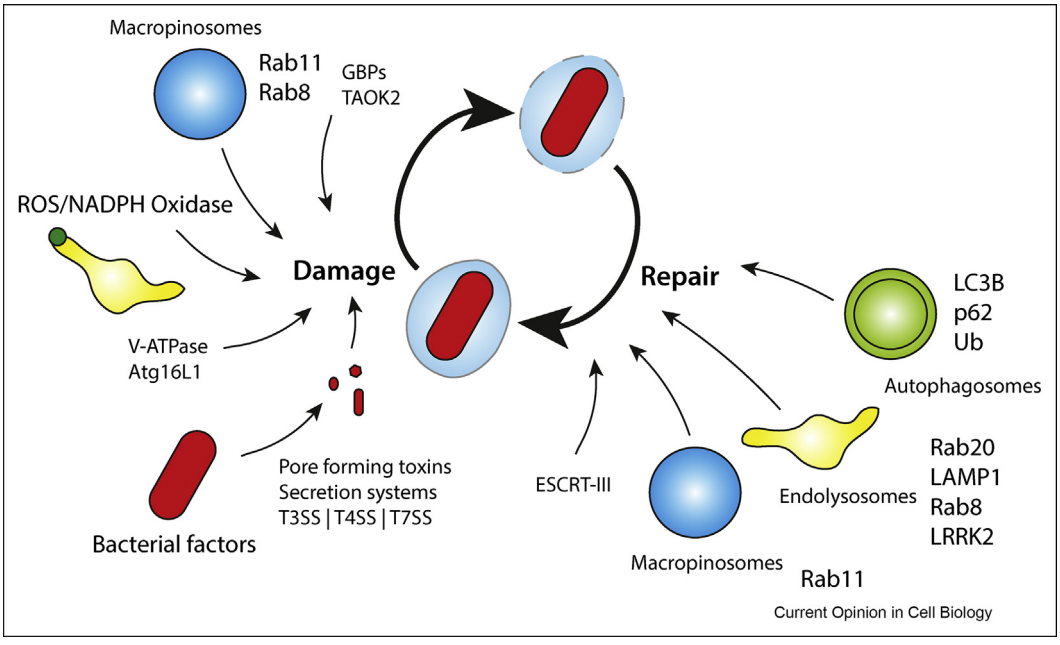
Regulation of BCV integrity is controlled by bacterial factors, such as the T3SS effector SopF that has been identified as first early BCV stabilizer for this pathogen acting antagonistically to SopB [31]. During BCV membrane damage avoidance, SopF also modifies the v-ATPase subunit ATP6V0C eventually blocking the induction of the host xenophagy response [68]. During these steps, SopF exploits host GTP-bound ADP-ribosylation factor (ARF) GTPases as co-factor required for the enzymatic ADP-ribosyltransferase activity of SopF on the vATPase. Eventually, the ATPase senses endomembrane damage to trigger selective autophagy [67]. The need to actively keep BCV membranes intact has also been recognized during *Legionella* infection leading to the vacuolar guard hypothesis [1]. A bacterial effector SdhA binds to OCRL an inositol 5-phosphatase controlling endosomal dynamics through the GTPase Rab5 and Rab11 blocking the pathway that has been described to promote vacuolar rupture in *Shigella* [2,11].

In summary, sensitive markers for the tracing of BCV membrane damage have revealed that a complex cross-talk of host and bacterial factors regulates the intracellular niche of bacterial pathogens.

The impact of switching intracellular niches for the pathogens

The different intracellular niches inhabited by the pathogens depend on various conditions, like the tissue context, the infected host cell type, as well as the cellular activation and metabolic status [8,12,32,37]. The changing intracellular microenvironments have

Figure 3



Host and pathogen factors that regulate switching between a membrane-bound compartment and the cytosol. Schematic representation of the different endomembrane damage and repair mechanisms. There is a dynamic interplay between the damaging of the phagosome (either by bacterial factors or for example through the production of ROS via the NADPH oxidase) and host mechanisms that promote the repair of the damaged bacterial-containing compartment. Some of the factors (such as interactions with macropinosomes) can contribute to both damage or repair.

important consequences in how the pathogen communicates with the cell, accesses nutrients, responds to metabolite signaling, and for the sensing of mechanical properties of the infected cell. Unique vacuolar and cytosolic virulence gene expression programs of the pathogens have been observed, for example *Salmonella* adapts to its specific intracellular niche. This illustrates how this archetypical vacuole-adapted pathogen requires extensive transcriptional reprogramming to successfully colonize the cytosol [49].

Even though the cytosol is assumed to be a more permissive environment rich in nutrients, it remains to be defined which specific intracellular niche is optimal for bacterial growth or restriction. Obtaining nutrients by vacuolar pathogens may require a high level of sophistication, such as massive membrane remodeling to generate *Salmonella*-induced filaments (SIFs). These membrane extensions increase the total surface of interactions with endocytic vesicles that fuse with the *Salmonella* containing vacuole and provide it with nutrients [33]. It is also conceivable that nutrients can be obtained within the BCV from the host inverting the T3SS to suck them up from the host cytosol, similar to extracellular *Escherichia coli* [43]. Pathogens adapt to host cell antimicrobial metabolic reprogramming. During infection of macrophages, bacteria are sensed in order shift the host metabolism from oxidative phosphorylation (OXPHOS) towards aerobic glycolysis. Consequently, *Salmonella* detects the accumulation of one of these metabolic intermediates, succinate, that trigger the induction of virulence factors important for survival [52]. Inversely, *Listeria* reprograms the host cell metabolism with consequences for intracellular trafficking. Mitochondrial respiration-dependent endocytic recycling is reduced in infected cells, in turn reducing bacterial infection by slowing the recycling of its host cell receptor c-Met [62]. Furthermore, mechanical forces within the intestinal epithelium can impact the infection process. During *Salmonella* host cell challenge, the pathogen induces local contraction forces that are related to inflammasome activation after sensing of the bacterial T3SS. Importantly, focal contractions precede, and are uncoupled from the death and expulsion of infected epithelial cells. This crosstalk involving mechanical forces allows the epithelium to increase cell numbers in infected areas [55].

Bacteria do not only damage the membrane of their own niche, but of the entire cell, hence plasma membrane damage regulation of host cells by bacterial factors is a relevant bacterial host subversion strategy. The Gasdermin family represents pore-forming cytolysins of eukaryotic cells, however they have recently been found to be subverted differently during infection. Gasdermin B (GSDMB) exhibits direct pore-forming microbiocidal activity through the recognition of phospholipids on the membranes of *Shigella* within the cytosol [21]. The T3SS effector protein IpaH7.8 targets human Gasdermin B (GSDMB) for ubiquitin-mediated proteolysis to protect *Shigella*. This circumvents pathogen destruction by natural killer cells via granzyme-A mediated activation of GSDMB [21]. Interestingly IpaH7.8 also contributes to species specificity by ubiquitinating human, but not mouse, Gasdermin D (GSDMD) and targeting it for proteasomal degradation [36].

It is possible that host cell death after membrane damage could also represent a niche switching strategy in case a secondary cell internalizes dead cells containing bacteria (e.g., efferocytosis). *Salmonella* infection can result in different forms of host cell death including apoptosis, pyroptosis, necroptosis and necrosis. The kind of cell death depends on the host cell type and *Salmonella* effectors, nevertheless pore-induced cell death and formation of structures named pore-induced intracellular traps (PIT) followed by efferocytosis by a secondary phagocyte (e.g. neutrophil or macrophage) could lead to generation of an efferosome comprising an apoptotic body or a PIT containing viable *Salmonella* [24]. This strategy functions as a Trojan horse strategy since PIT-related membranes containing *Salmonella* protect the bacteria from ROS in neutrophils [23].

Internalized bacteria conquering their specific niches are capable to avoid delivery of antimicrobial molecules to the compartment they are localized [6]. The bactericidal role of the small host metabolite itaconate has been characterized during *Legionella* and mycobacterial infection [40,41]. In the case of *Salmonella*, Rab32 a GTPase known for its primordial importance in species-specific restriction interacts with the itaconate synthesizing enzyme aconitate decarboxylase 1 (IRG1) for its delivery to the bacterial compartment [10] This mechanism may also play a role in the restriction of other pathogens, such as *Staphylococcus aureus* or *Candida albicans* [3]. Delivery of antibiotics to intracellular bacteria is an important issue as some of them tend to accumulate in specific organelles such as lipid droplets and changing lipid droplet numbers affects the efficacy of the antibiotic bedaquiline [20]. *In vivo*, bedaquiline accumulates in polymorphonuclear cells and foamy macrophages, suggesting that cell type specific accumulation of antibiotics contributes to antibiotic efficacy [18]. Intracellular localization in different niches also differentially affect mycobacteria, for example membrane-bound bacteria are more susceptible to the antibiotic PZA that have a mode of action that requires acidic pH [57]. Ideally, defining combined therapies that target a maximum of intracellular niches should be sought.

Niche switching, recognition by the host and avoidance by the pathogen

The innate immune response needs to sense intracellular bacterial pathogens in their different niches, and the response needs to be dosed depending on the threat

and localization. Recognition of vacuolar bacteria is of paramount importance as all cell invaders travel through this compartment at first after internalization. The phagosomal or BCV membrane separate the bacteria and their pathogen-associated molecular patterns (PAMPs) from the cytosol. Nevertheless, vacuolar bacteria can stimulate cytosolic pattern recognition receptors (PRRs) such as TLRs affecting the infection process, such as phagosome maturation [44]. As more and more bacteria are recognized to be switching between membrane bound and cytosolic localization, research on niche-dependent host cell recognition has increased. It remains unclear how cell wall components from live vacuolar bacteria are transported to the cytosol. Potentially, bacteria actively transfer nucleic acids to the cytosol, or continuous damage/deficient repair of the phagosomal membrane contributes to access.

Recognition of cytosolic bacteria is critical for effective immune responses. Recently, LPS has been shown to be directly targeted by untypical ubiquitylation through the core domain of the E3 ligase RNF213 (Otten et al., 2021). This represents an antibacterial cell autonomous immune response, as cells lacking the RNF213 ligase do not exhibit antibacterial autophagy. Furthermore, the members of the family of dynamin-related guanylate binding proteins (GBPs) are important regulators of the innate immune response to intracellular bacteria. Human GBP1 acts as an LPS-binding surfactant that destabilizes the rigidity of the outer membrane, impairing actin-driven dissemination of *Shigella* [30]. In epithelial cells, GBP1 also functions as LPS sensor in the cytosol and assembles a platform for caspase-4 recruitment and activation at LPS-containing membranes as the first step of non-canonical inflammasome signaling [56]. In response to cytosol-invading bacteria, activation of caspase-4 through the GBP platform is essential to induce GSDMD-dependent pyroptosis and processing of interleukin-18, thereby destroying the replicative niche for intracellular bacteria and alerting neighboring cells, respectively [64].

Septins are cytoskeleton-associated proteins implicated in multiple cell functions. They recognize cytosolic bacteria and entrap them within cage-like structures. Cardiolipin, a curvature-specific phospholipid, promotes septin recruitment to highly curved membranes of *Shigella*, and bacterial mutants lacking cardiolipin exhibit less septin cage entrapment [28]. Septin cages could be reconstituted providing additional insights on how these structures are formed through an amphipathic helix on SEP6 that senses positively curved bacterial membranes (Lobato-Marquez et al., 2021). Intracellular entrapment by septins further requires mitochondria, as mitochondrial fragmentation reduces septin cage formation, allowing *Shigella* proliferation [60]. Septin cages and GBP1 bacterial recognition have been proposed to represent complementary strategies to limit *Shigella* dissemination highlighting redundant mechanisms for the control of bacterial proliferation, and [29]. It is interesting to note that GBPs (as well as RNF213) are regulated by interferons, which is not that case for septins pointing towards the importance of the activation status of infected cells for the differential triggering of antimicrobial processes. The intracellular pathogen *Rickettsia parkeri* possesses protein-lysine methyltransferases that actively modify their outer membrane proteins (OMPs) and prevent their ubiquitylation. This suggests lysine methylation as an important factor of rickettsial intracellular survival for the shielding of bacterial proteins from ubiquitylation to evade autophagic targeting [15,16].

Outlook and perspectives

The conditioned niche concept for intracellular pathogens underscores the need to revisit established dogma of the host–pathogen crosstalk. Its formulation has become possible because of techniques that provide precise information on the underlying dynamics as well as resolution at the level of molecules. Advances in cell biology methods have revealed that many pathogens previously considered to live exclusively in a phagosome or BCV are now reported to access the cytosol [35]. Imaging approaches that comprise simultaneous information on both, single pathogens and single host cells are needed to define in time and space bacterial niches. Correlative large volume approaches that combine fluorescence and electron microscopy have proven to be very informative, also capturing more recently the attention of cell biologists in general [65]. Higher resolution can be gained using *in cellulo* structural biology methods at single molecule resolution in combination with optical imaging that emerges as powerful tool for the study of intracellular niche formation [5]. It is foreseeable that these technologies can be further developed integrating subcellular proteomics. The conditioned niche concept also requires to experimentally capture within a given host the different habited niches by one specific pathogen. As we encounter niche diversity it is important to investigate the variable interactions within different host cell types and responses to specific microenvironments. There is a pressing need of a consequent integration of novel tools for screening such as CRISPR/Cas9 and gene trapping with organoids and tissue bioengineering to recreate more physiological environments [45]. Novel approaches such as *in vitro* reconstitution of membrane trafficking targeting will help to better understand the molecular mechanisms of pathogen subversion at a quantitative level. One of the main challenges to study niche switching *in vivo* is the heterogeneity at the single cell level. In this context, using bacterial reporters in combination with high resolution 3D imaging will be critical. Also, the use of small animal models like zebrafish that are amenable to single cell tagging and imaging. Overall, high resolution correlative approaches with labels in both host and

pathogen that capture different layers of biological information will be crucial.

Conflict of interest statement

Nothing declared.

Acknowledgements

Work in the Host pathogen-interactions in tuberculosis lab is supported by the Francis Crick Institute (to MGG), which receives its core funding from Cancer Research UK (FC001092), the UK Medical Research Council (FC001092) and the Wellcome Trust (FC001092). This research was funded in whole, or in part, by the Wellcome Trust (FC001092). The Dynamics of Host Pathogen Interaction Unit acknowledges funding from the Institut Pasteur, the European Union (ERC-CoG "Endosubvert"), and the Agence National pour la Recherche (Program "HBP Sensing", "PureMagRupture"). Furthermore, the JE team is supported by the LabExes "Milieu Interieur" and "IBEID". For the purpose of Open Access, the author have applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

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