The History of Septin Biology and Bacterial Infection

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ABSTRACT

Investigation of cytoskeleton during bacterial infection has significantly contributed to both cell and infection biology. Bacterial pathogens *Listeria monocytogenes* and *Shigella flexneri* are widely recognized as paradigms for investigation of the cytoskeleton during bacterial entry, actin-based motility and cell-autonomous immunity. At the turn of the century, septins were a poorly understood component of the cytoskeleton mostly studied in the context of yeast cell division and human cancer. In 2002, a screen performed in the laboratory of Pascale Cossart identified septin family member MSF (MLL septin-like fusion, now called SEPT9) associated with *L. monocytogenes* entry into human epithelial cells. These findings inspired the investigation of septins during *L. monocytogenes* and *S. flexneri* infection at the Institut Pasteur, illuminating important roles for septins in host-microbe interactions. In this review, we revisit the history of septin biology and bacterial infection, and discuss how the comparative study of *L. monocytogenes* and *S. flexneri* has been instrumental to understand septin roles in cellular homeostasis and host defense.

1. INTRODUCTION

Bacterial interactions with the actin cytoskeleton

Rearrangements of the host cell cytoskeleton during infection are well known to promote events that benefit a bacterial pathogen, including internalization into the host cell and dissemination from cell-to-cell (Haglund and Welch, 2011). Over the past ~30 years, *Listeria monocytogenes* has emerged as a model bacterial pathogen used to study cytoskeleton rearrangements during infection, providing valuable insights into fundamental aspects of host cell biology (Radoshevich and Cossart, 2018). For example, *L. monocytogenes* has been critical to reveal a role for clathrin in bacterial invasion (Veiga and Cossart, 2005), and a role for the actin-related-protein 2/3 (ARP2/3) complex in actin-based motility (Welch *et al.*, 1997).

Invasive bacteria promote their internalization into normally non-phagocytic host cells using two different mechanisms referred to as ‘‘zippering’’ and ‘‘triggering’’ (Cossart and Sansonetti, 2004). Zippering bacteria (such as *L. monocytogenes*) present invasion molecules that mimic endogenous ligands to host cell surface receptors, initiating signaling cascades that result in actin polymerization and membrane extensions which zipper around invading bacteria. Triggering bacteria (such as *S. flexneri*) rely on their type III secretion system (T3SS) to deliver effector proteins into the host cell cytosol that trigger actin polymerization and membrane ruffling, engulfing invading bacteria in a process similar to macropinocytosis.

Following invasion, bacterial pathogens are enclosed within an internalization vacuole (called the phagosome). In some cases, bacteria such as *L. monocytogenes* and *S. flexneri* escape to the host cell cytosol where they replicate and initiate actin-based motility by polymerization of actin at one pole of the bacterium (Welch & Way, 2013). In this way, actin-based motility enables cytosolic bacterial pathogens to escape cell-autonomous immunity and disseminate from cell-to-cell.

The septin cytoskeleton

As compared to actin, relatively little was known about the role of septins during bacterial infection. Septins were discovered by Nobel Laureate Leeland Hartwell while screening for cell division cycle (cdc) mutants in the budding yeast *Saccharomyces cerevisiae* (Hartwell, 1971). Septins have since been identified in all eukaryotes (except higher plants) and are considered a component of the cytoskeleton interacting with actin, microtubules and cellular membrane (Mostowy and Cossart, 2012). In humans, septins are classified into 4 groups (namely the SEPT2, SEPT3, SEPT6 and SEPT7 groups) based on sequence homology. Septin subunits from different groups interact through their G (consisting of the GTP-binding domain) and NC (consisting of the amino and carboxy-terminal regions) interfaces, forming non-polar complexes and filaments. Septin filaments can bundle and by interacting with cellular membrane form higher-order structures, such as rings.

Investigations on septin molecular functions have highlighted their roles in numerous biological processes, including cell division and motility, by acting as scaffolds for protein recruitment and diffusion barriers for subcellular compartmentalization (Bridges and Gladfelter, 2015). Recent studies have illuminated septin roles in the organization of actin and microtubule networks (Spiliotis, 2018). In agreement with septins being key for cellular homeostasis, septin dysfunction has been implicated in a wide variety of pathological conditions including cancer, male infertility and neurodegenerative disorders (Dolat, Hu, & Spiliotis, 2014). A role for septins in bacterial infection was first suggested from studying *L. monocytogenes* invasion ~20 years ago in the laboratory of Pascale Cossart (Pizarro-Cerdá *et al.*, 2002). Since then, septins have been associated with a wide variety of bacterial pathogens, including *Chlamydia trachomatis*, *Clostridium difficile*, *Salmonella* Typhimurium and enteropathogenic *Escherichia coli* (Volceanov *et al.*, 2014; Nölke *et al.*, 2016; Lee *et al.*, 2017; Boddy *et al.*, 2018), and play important roles in host cell infection (Torraca and Mostowy, 2016; Van Ngo and Mostowy, 2019) **(Figure 1)**. In this review, we discuss how the study of *L.*

monocytogenes and *S. flexneri* at the Institut Pasteur launched septins into the field of bacterial infection.

2. SEPTINS AND BACTERIAL ENTRY

Actin rearrangements during bacterial entry

How *L. monocytogenes* can enter non-phagocytic cells has been investigated in great detail (Cossart and Sansonetti, 2004; Radoshevich and Cossart, 2018). Several surface proteins contribute to this process, including Internalin (InlA) which promotes bacterial entry through interaction with human E-cadherin, a transmembrane glycoprotein located at adherens junctions and interacting with actin through catenins (Bonazzi et al., 2009; Gaillard et al., 1991). A second *L. monocytogenes* invasion protein is Internalin B (InlB) which interacts with hepatocyte growth factor receptor (HGF-R / Met), a transmembrane receptor tyrosine kinase (Shen et al., 2000). Both the InlA- and InlB-mediated entry pathways require activation of the ARP2/3 complex, inducing localized polymerization of actin to promote bacterial engulfment.

Septin roles in bacterial entry: from recruitment to function

To identify new players in *L. monocytogenes* entry, the laboratory of Pascale Cossart used a proteomic approach based on isolation of internalization vacuoles containing InlA- or InlB-coated latex beads (Pizarro-Cerdá *et al.*, 2002). From this, it was discovered that septin family member MSF (MLL septin-like fusion), now referred to as SEPT9 (Macara *et al.*, 2002), is enriched at the site of InlB-mediated entry. Subsequent experiments using fluorescent microscopy showed that SEPT9 is recruited to the entry site of InlB-coated beads, where it co-localizes with actin. These observations suggested that SEPT9 regulates the InlB-mediated entry of *L. monocytogenes*. Consistent with this, studies using bacterial cells

revealed that SEPT9, and its interacting partners SEPT2 and SEPT11, are recruited as 0.6 µm collar-like structures with actin at the entry site of *L. monocytogenes* (Mostowy, Tham, et al., 2009b) **(Figure 2 L1)**. Septin collars were similarly detected at the entry site of triggering bacteria, including *S. flexneri* **(Figure 2 S1)**. Considering that septins function as hetero-oligomers and the position of SEPT9 in the human septin complex was not yet clear, studies initially focused on SEPT2 because it was viewed as central for septin complex formation and function in human cells (Sirajuddin *et al.*, 2007). The depletion of SEPT2 using small interfering RNA (siRNA) significantly decreased the entry of *L. monocytogenes* and *S. flexneri*, revealing that SEPT2 can promote bacterial entry. Surprisingly, a separate study showed that SEPT11 restricts InlB-mediated entry by *L. monocytogenes*, highlighting that different septins may have different roles during bacterial entry (Mostowy et al., 2009a).

Importantly, treatment of cells with cytochalasin D (an inhibitor of actin polymerization) impaired septin recruitment to the site of bacterial entry, indicating that actin polymerization precedes septin assembly. Using fluorescence resonance energy transfer (FRET), it was shown that SEPT2 can activate PI 3-kinase signaling during InlB-Met interactions (Mostowy et al., 2009a). Using atomic force microscopy (AFM), it was shown that septins anchor Met to the cortical actin cytoskeleton, where it may regulate surface receptor dynamics (Mostowy et al., 2011a). At the plasma membrane, septins may act as a diffusion barrier to restrict the localization of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2)-enriched microdomains crucial for signalling processes (Sharma *et al.*, 2013). Recent work using *Salmonella* Typhimurium showed that septins are involved in membrane ruffle morphology and signalling through the actin nucleating protein FHOD1 (Boddy *et al.*, 2018). Taken together, the septin cytoskeleton can be viewed as a molecular platform modulating cytoskeletal dynamics and signal transduction events during bacterial entry. These conclusions are in agreement with work showing that septins assemble into collar-like structures at the base of the phagocytic cup during FcγR-mediated phagocytosis in macrophages and neutrophils (Huang et al., 2008).

3. SEPTINS AND ACTIN-BASED MOTILITY

The paradigm of the bacterial actin tail

The observation that septins form collar-like structures around invading bacteria initiated great interest in the role of septins during other actin-based strategies exploited by intracellular pathogens, such as actin-based motility. Intracellular bacterial movement was first observed in the 1950s in cells infected with *Rickettsia rickettsia*, the causative agent of Rocky Mountain spotted fever (Schaechter *et al.*, 1957), and direct evidence for bacterial actin-based motility came in the 1980s from cells infected with *L. monocytogenes* (Tilney and Portnoy, 1989) and *S. flexneri* (Bernardini et al., 1989). In the case of *L. monocytogenes,* actin tail formation requires expression of the surface protein ActA at the bacterial cell pole, which acts as a mimic of the nucleation promoting factor Wiskott-Aldrich syndrome protein (WASP) to recruit and activate the ARP2/3 complex (Welch et al., 1997). In contrast, *S. flexneri* expresses the bacterial autotransporter protein IcsA (also called VirG), whose activity recruits the neural Wiskott-Aldrich syndrome protein (N-WASP) and consequently ARP2/3 to the bacterial cell pole (Bernardini et al., 1989; Makino et al., 1986). Polar localization of IcsA is essential for actin tail formation, and a recent study showed that polymerization of MreB (the bacterial actin homologue) promotes polar IcsA positioning for actin tail formation (Krokowski *et al.*, 2019).

Actin-based motility is used by various pathogens for dissemination within and between cells (Gouin, Welch, & Cossart, 2005; Welch & Way, 2013). However, infected host cells have powerful mechanisms to restrict actin-based motility, highlighting unforeseen aspects of cell-autonomous immunity.

Enter the *Shigella* septin cage

Consistent with studies showing that septins are recruited to actin rich sites of bacterial entry, septins form ring-like structures around approximately 25% of *L. monocytogenes* and *S. flexneri* actin tails (Mostowy *et al.*, 2010) **(Figure 2 L2 and S2)**. The depletion of SEPT2 or SEPT9 had no significant impact on bacterial directionality or movement, indicating that septin recruitment is not essential for actin-based motility. In agreement with this, septins were not detected among proteins identified by mass-spectrometry of *L. monocytogenes* actin tails (Van Troys *et al.*, 2008). Importantly, *L. monocytogenes* Δ*actA* and *S. flexneri* Δ*icsA*, isogenic mutants unable to polymerize actin tails, both fail to recruit septin (Mostowy *et al.*, 2010). Together, these experiments suggested that septin rings assemble at sites of actin polymerization and depend on actin polymerization.

Strikingly, cytosolic *S. flexneri* surrounded by an actin cloud, though without an actin tail, are surrounded by septin cage-like structures **(Figure 2 S5)**. In this case, septin cage-like structures compartmentalize approximately 15-30% of cytosolic *S. flexneri* (Mostowy *et al.*, 2010). SEPT2 or SEPT9 depletion significantly increased the number of *S. flexneri* with actin tails, indicating that septins restrict actin tail formation. Similarly, inhibiting myosin II function using siRNA or blebbistatin increased the number of *S. flexneri* with actin tails. Treatment of cells with Tumour Necrosis Factor α (TNFα), a pleiotropic cytokine shown to stimulate association of myosin II to SEPT2, promotes septin caging and restricts actin tail formation (Mostowy *et al.*, 2010). These results highlight septin caging as a host defense mechanism counteracting actin-based motility to restrict the dissemination of *S. flexneri*. Since its discovery in *S. flexneri*-infected tissue culture cells, septin cage-like structures have been observed *in vivo* in *S. flexneri*-infected zebrafish larvae (Mostowy *et al.*, 2013), and have also been observed in tissue culture cells infected with *Mycobacterium marinum* (Mostowy *et al.*, 2010), enteropathogenic *Escherichia coli* (EPEC) (Lee *et al.*, 2017) and vaccinia virus (Pfanzelter *et al.*, 2018). In the case of vaccinia virus, septins exert their antiviral effect by forming rings around virions at the plasma membrane to suppress their release from infected cells.

Discovery of the *S. flexneri* septin cage revealed a new role for the cytoskeleton in cell-autonomous immunity by inhibiting actin-based motility. Since then, another mechanism inhibiting actin-based motility has been described: the coating of cytosolic *S. flexneri* by the interferon-inducible GTPase family of guanylate-binding proteins (GBPs) (Li *et al.*, 2017; Wandel *et al.*, 2017; Piro *et al.*, 2017). In this case, *S. flexneri* antagonizes GBP-mediated cellular defenses by secreting the E3 ubiquitin ligase IpaH9.8, which ubiquitinates and degrades GBPs in a proteasome-dependent manner. In the future, it will be of great interest to explore the relationship between septin cages and GBP coats.

Why do only a fraction of cytosolic *S. flexneri* recruit septins? Considering that bacterial pathogens often manipulate host cell proteins and processes to evade cell-autonomous immunity (Huang and Brumell, 2014), it is highly likely that *S. flexneri* has evolved mechanisms to evade septin cage entrapment. For example, post-translational modification of host cell proteins by bacterial pathogens is well known to interfere with protein activity (Salomon and Orth, 2013). Septins are regulated by a variety of post-translational modifications including acetylation, phosphorylation, ubiquitination and sumoylation (Koch et al., 2015). New work has shown that the *S. flexneri* effector IcsB is an 18-carbon fatty acyltransferase catalysing the lysine N_{ε}-fatty acylation of SEPT7 and SEPT11 (Liu *et al.*, 2018). Whether post-translational modifications may affect septin cage assembly, and whether bacteria can interfere with this process, remains to be determined.

4. OTHER SEPTIN ROLES IN CELL-AUTONOMOUS IMMUNITY

Septins and autophagy

Autophagy delivers cytosolic material enclosed in a double-membraned vacuole (called the autophagosome) to the lysosome for degradation (Levine *et al.*, 2011). In this way, autophagy acts as a cytosolic quality control mechanism, eliminating protein aggregates, damaged

organelles and intracellular microbes to maintain cellular homeostasis. Targeting of bacteria to autophagy can proceed through ubiquitin-dependent and -independent signaling pathways (Khaminets *et al.*, 2016). Ubiquitinated bacteria are recognized by autophagy receptors such as p62 (sequestosome 1 or SQSTM1) and NDP52 (nuclear dot protein, 52 kDa), pattern recognition receptors called sequestosome 1/p62-like receptors (SLRs) that recruit membranes for autophagosome formation through their interaction with ATG8 family proteins such as LC3B (Johansen and Lamark, 2019). Targeting of bacteria can also proceed through ubiquitin-independent pathways, such as LC3-associated phagocytosis (LAP) or opsonization by complement (Mostowy, 2013; Sorbara *et al.*, 2018).

However, many intracellular bacteria have mechanisms to avoid recognition by autophagy (Huang & Brumell, 2014). In the case of *L. monocytogenes*, avoidance of autophagy is mediated via ActA by promoting recruitment of actin-polymerization machinery on the bacterial surface, and also by enabling bacteria to move away from autophagic membranes via actin-based motility (Cheng et al., 2018; Yoshikawa et al., 2009) **(Figure 2 L3)**. In addition to ActA, *L. monocytogenes* phospholipase C (PLC) can reduce the intracellular levels of phosphatidylinositol 3-phosphate (PtdIns3P), a lipid required for LC3B lipidation (Tattoli *et al.*, 2013; Mitchell *et al.*, 2015). In the case of *S. flexneri*, avoidance of autophagy is mediated via the bacterial effector protein IcsB, which prevents the recruitment of ATG5 (a protein critical for autophagosome maturation) to IcsA (Ogawa *et al.*, 2005). A more recent study discovered that N_{ε}-fatty acyltransferase activity of IcsB modifies CHMP5 (a component of the ESCRT-III complex) to escape from autophagy (Liu *et al.*, 2018). In addition to IcsB, *S. flexneri* VirA can inactivate Rab1 to counteract autophagy (Dong *et al.*, 2012).

Several autophagy markers (e.g., p62, NDP52 and LC3B) are recruited to septin cages (Mostowy *et al.*, 2010; Mostowy *et al.*, 2011b) **(Figure 2 S6)**, and *S. flexneri* IcsB mutants are compartmentalized in septin cages more efficiently than the wild-type strain (Mostowy *et al.*, 2010), consistent with a role for IcsB in autophagy escape (Ogawa *et al.*, 2005; Liu *et al.*,

2018). Strikingly, when septins (SEPT2 or SEPT9) or autophagy components (p62, ATG5, ATG6 or ATG7) are depleted by siRNA, both septin cages and autophagy markers fail to recruit around *S. flexneri* (Mostowy *et al.*, 2010). These results highlight an interdependence between septin assembly and autophagy. In agreement with this, a study using proteomics found autophagy and mitochondrial proteins associated with septins in *S. flexneri*-infected cells **(Figure 2 S4)**, and that actin-polymerizing *S. flexneri* fragment mitochondria to escape from septin cage-mediated autophagy (Sirianni *et al.*, 2016).

In a first study to investigate bacterial autophagy *in vivo*, infection of zebrafish larvae with *S. flexneri* highlighted autophagy as a crucial component of innate immunity at the whole animal level (Mostowy *et al.*, 2013). Here, depletion of p62 using morpholino oligonucleotide significantly reduced the number of septin cage-associated *S. flexneri*, and significantly increased bacterial burden. In agreement with the view that septin cages are anti-bacterial, work using HeLa cells showed that approximately 50% of *S. flexneri* entrapped in septin cages are not metabolically active (Sirianni *et al.*, 2016). On the other hand, septins and the autophagy machinery can regulate metabolic pathways that promote the proliferation of intracellular *S. flexneri* not entrapped in septin cages (Lobato-Márquez et al., 2019).

Although the fate of *S. flexneri* in septin cages is relatively well characterized, how septins sense cytosolic bacteria was mostly unknown. It has been recognized for approximately 10 years that septin assembly is membrane facilitated (Tanaka-Takiguchi *et al.*, 2009), and recent work suggested that septins sense micron-scale curvature at the plasma membrane (Bridges et al., 2016). In agreement with this, a recent study showed that septins are recruited to regions of micron-scale curvature presented by dividing bacterial cells **(Figure 2 S3)**, and cardiolipin (a curvature-specific phospholipid) promotes septin recruitment to these regions (Krokowski *et al.*, 2018). Following pharmacological inhibition of bacterial cell division, septins are recruited to bacterial cell poles but fail to assemble into cages, indicating that bacterial cell growth is required for septin cage entrapment. Therefore, bacterial cell division can be viewed

as a fundamental danger signal used by the host cell to recognize intracellular bacteria (Krokowski *et al.*, 2018; Van Ngo and Mostowy, 2019; Krokowski and Mostowy, 2019).

Septins and inflammation

A growing number of studies have indicated that the cytoskeleton plays key roles during inflammation (Mostowy and Shenoy, 2015). For example, actin has been linked to inflammation control through regulation of the NACHT, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome, a large multimeric complex that activate caspase-1 and pro-inflammatory cytokines (Burger et al., 2016). Following cellular damage or infection, inflammasome formation and pyroptosis (an inflammatory type of programmed cell death) is linked with disruption of actin and microtubules (Davis *et al.*, 2019). Although a role for septins in inflammasome activity has not yet been shown, recent studies have linked septins to dysregulated inflammatory responses (Lee *et al.*, 2017; Mazon-Moya *et al.*, 2017). In the case of human monocytic THP-1 cells infected with *S. flexneri*, disruption of WASP by short hairpin RNA (shRNA) reduced septin caging and increased inflammasome activity and host cell death (Lee *et al.*, 2017). At the whole animal level, work using the *S. flexneri*-zebrafish infection model discovered an *in vivo* role for septins in inflammation control (Mazon-Moya *et al.*, 2017). Here, depletion of Sept7b or Sept15 (zebrafish orthologues of human SEPT7) using morpholino oligonucleotide significantly increased host susceptibility to *S. flexneri* infection. Moreover, Sept15 morphants exhibit significantly increased caspase-1 activity and host cell death, indicating that septins restrict inflammation *in vivo*. Taken together, these results suggest that septins may interact with the inflammasome, and factors important in septin-mediated restriction of inflammation can represent novel drug targets for the control of bacterial infection and inflammatory disorders.

5. CONCLUSION AND FUTURE PERSPECTIVES

The study of infection by *L. monocytogenes* and *S. flexneri* has significantly advanced our understanding of cellular microbiology and cell-autonomous immunity. *In vitro* reconstitution of *L. monocytogenes* and *S. flexneri* actin-based motility has been essential to identify key proteins underlying actin polymerization (Loisel et al.,, 1999;Welch et al., 1998). Similarly, *in vitro* reconstitution of the *S. flexneri* septin cage may yield important insights on the biochemical, molecular and structural aspects of septin-microbe interactions. To elucidate the precise molecular basis of *S. flexneri* septin caging, the use of high resolution (such as cryo-electron tomography) and high content (to screen host and/or bacterial factors) microscopy techniques will be of great interest. Although this review is focused on *L. monocytogenes* and *S. flexneri* infection, it is essential to understand the full breadth of microbial pathogens regulated by septin biology, and to test how widespread is the role of cell shape and membrane curvature in pathogen sensing (Torraca and Mostowy, 2016; Van Ngo and Mostowy, 2019). Our current knowledge of septin function during infection mostly derives from work performed *in vitro* using non-phagocytic cells (e.g. HeLa); future work should address septin function in immune cells (e.g. macrophages, neutrophils) and whole animal models (e.g. zebrafish and mice). Overall, the study of septin biology and bacterial infection has provided unexpected insights into septin biology, and septin recruitment by a wide range of pathogens will likely continue to highlight novel septin functions. This information may help to elucidate therapeutic treatments for infection, inflammation and other human diseases in which septins have been implicated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

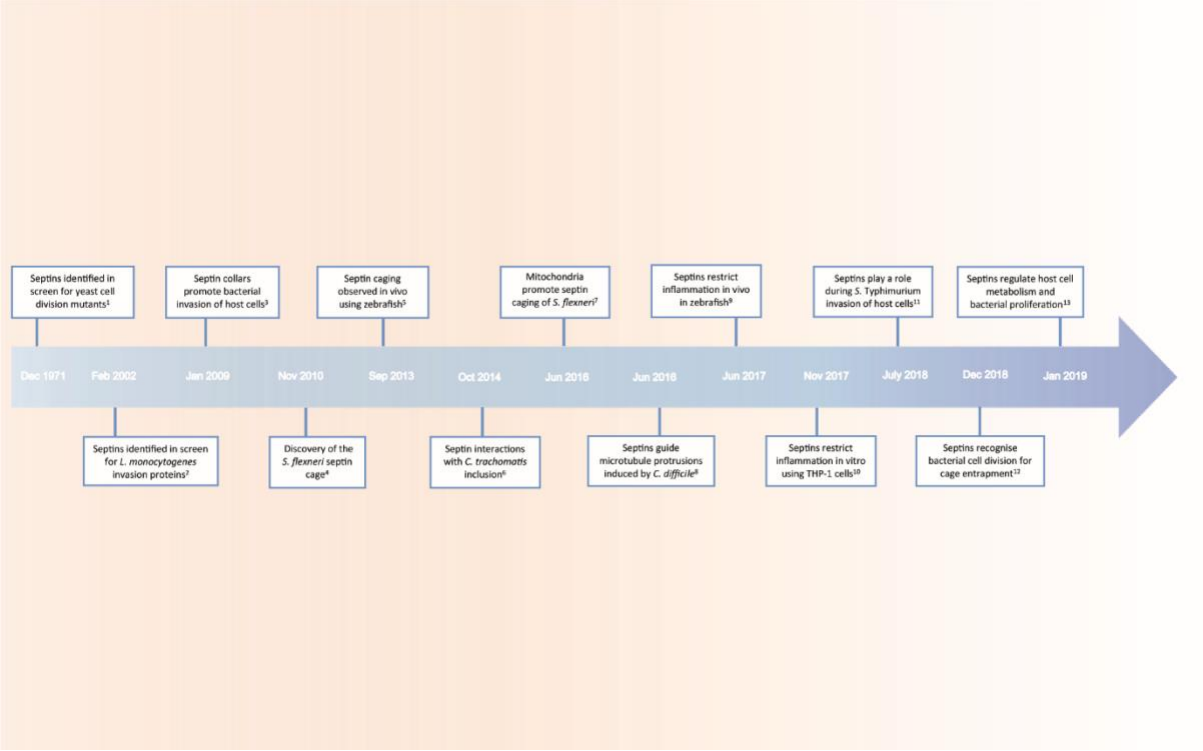

Figure 1. Timeline: A history of septin biology and bacterial infection

1) Septins were discovered using mutagenesis screens in yeast to find genes crucial for cell division (Hartwell, 1971); 2) In the case of infection biology, septins were discovered at the Institut Pasteur using a proteomic screen to find genes crucial for *L. monocytogenes* entry (Pizarro-Cerdá *et al.*, 2002); 3) Septins assemble as collar-like structures around invading bacteria, launching septins into the field of bacterial infection (Mostowy et al., 2009b); 4) Septins assemble into cage-like structures around actin-polymerizing *S. flexneri*, revealing a new link between the cytoskeleton and cell-autonomous immunity (Mostowy *et al.*, 2010); 5) Septin caging observed *in vivo* using *S. flexneri* infection of zebrafish, a first animal model testing the role of bacterial autophagy *in vivo* (Mostowy *et al.*, 2013); 6) Septins organize filamentous actin around the *C. trachomatis* inclusion, and are required for inclusion release by extrusion (Volceanov *et al.*, 2014); 7) Mitochondria promote septin assembly into cages that entrap *S. flexneri* for targeting to autophagy, a first report to highlight septin-mitochondria interactions (Sirianni *et al.*, 2016); 8) Septins guide microtubule protrusions induced by *C. difficile* during host cell adhesion (Nölke *et al.*, 2016); 9) Septins discovered to restrict

inflammation *in vivo* and protect zebrafish larvae from *S. flexneri* infection (Mazon-Moya *et al.*, 2017); 10) Septin-mediated autophagy in THP-1 cells observed to restrict inflammation *in vitro* (Lee *et al.*, 2017); 11) Septins regulate actin dynamics to promote *S.* Typhimurium entry in host cells (Boddy *et al.*, 2018); 12) Septins recognize micron-scale membrane curvature of dividing *S. flexneri* for delivery to lysosome, highlighting a new link between cell division and cell-autonomous immunity (Krokowski *et al.*, 2018); 13) Septins and the autophagy machinery can promote the proliferation of intracellular *S. flexneri* not entrapped in septin cages (Lobato-Márquez *et al.*, 2019).

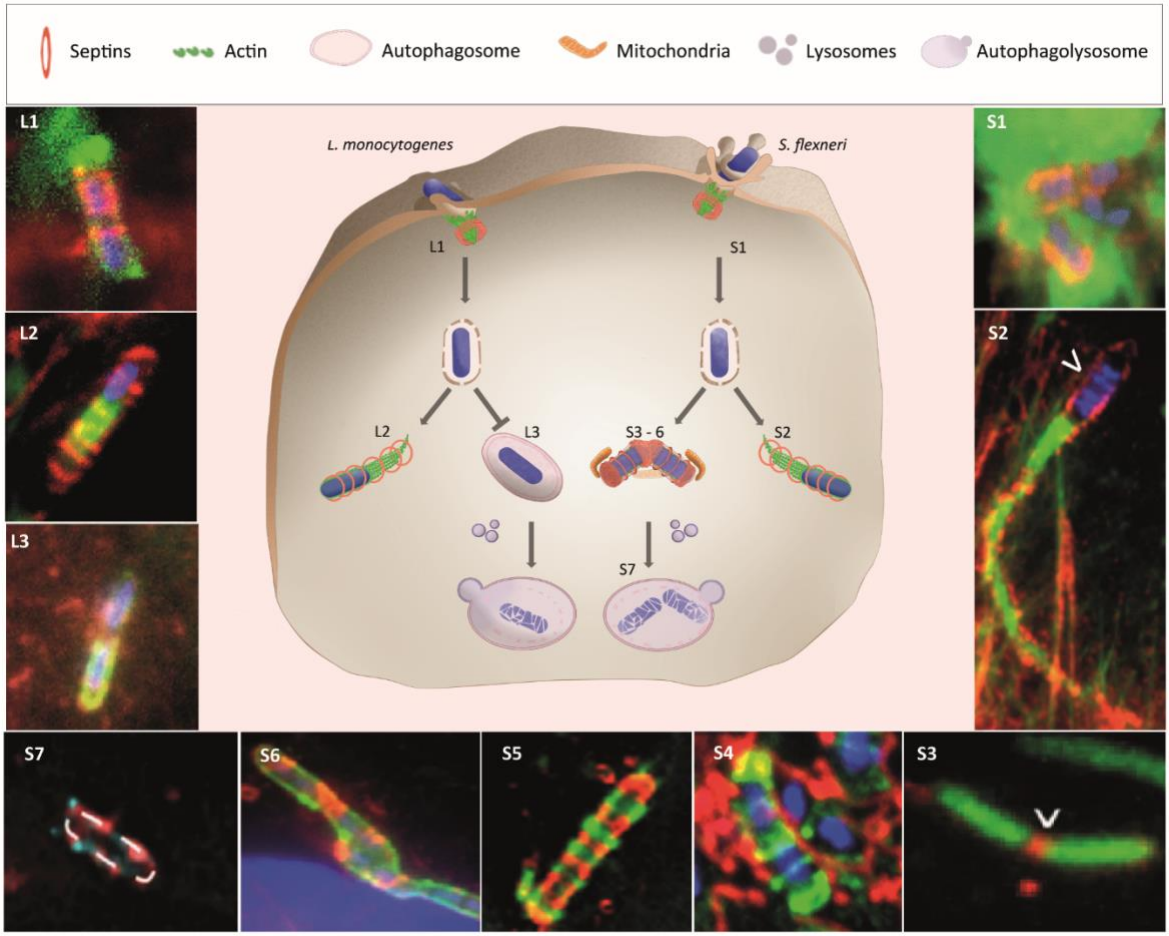

Figure 2. *L. monocytogenes* and *S. flexneri* interactions with the septin cytoskeleton

(Center) Model illustrating the interaction of *L. monocytogenes* (left) and *S. flexneri* (right) with septins during infection of host cells. (Surrounding) Representative immunofluorescence

micrographs of septin-bacteria interactions. (L1, S1) Septin recruitment to the site of *L. monocytogenes* and *S. flexneri* entry in Jeg-3 and HeLa cells, respectively. Endogenous septin stained with anti-SEPT9 (*L. monocytogenes*) or anti-SEPT2 (*S. flexneri*), actin stained with anti-F-actin (green), and bacteria marked using DAPI (blue). Images adapted with permission from (Mostowy *et al.*, 2009b). (L2, S2) Septin recruitment to actin tail of *L. monocytogenes* and *S. flexneri* in Jeg3 cells and HeLa cells, respectively. Endogenous septin stained with anti-SEPT9, actin stained with phalloidin (green), and bacteria marked using DAPI (blue). Images adapted with permission from (Mostowy *et al.*, 2010). (L3) Recruitment of autophagy markers to *L. monocytogenes* Δ*actA* in HeLa cells. p62 stained with anti-p62 (green), NDP52 stained with anti-NDP52 (red), and bacteria marked using DAPI (blue). Image adapted with permission from (Mostowy et al., 2011b). (S3) Septins are recruited to micron-scale curvature of dividing intracellular bacteria. SEPT6-GFP HeLa cells were infected with *S. flexneri* mCherry. White arrowheads indicate septin recruitment to the bacterial division site. Image adapted with permission from (Krokowski *et al.*, 2018). (S4) Mitochondria promote septin cage assembly. HeLa cells stably expressing SEPT6-GFP were transfected with Mito-BFP and infected with *S. flexneri* mCherry. SEPT6 is shown in green, mitochondria in red and *S. flexneri*-mCherry in blue. Image adapted with permission from (Sirianni *et al.*, 2016). (S5) The *S.flexneri*-septin cage. Endogenous septin stained with anti-SEPT9, actin stained with phalloidin (green), and *S. flexneri* marked using DAPI (blue). Image adapted with permission from (Mostowy *et al.*, 2010). (S6) Septin caged bacteria are targeted to autophagy in HeLa cells expressing ATG8-GFP. Endogenous septin stained with anti-SEPT2 (red) and *S. flexneri* marked using DAPI (blue). Image adapted with permission from (Mostowy *et al.*, 2010). (S7) Septins target bacteria to lysosomal degradation. SEPT6-GFP HeLa cells labelled with LysoTracker red. Septin cage-entrapped *S. flexneri* is shown as dashed white outline. Image adapted with permission from (Krokowski *et al.*, 2018).