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REVIEW

SUBJECT COLLECTION: CELL BIOLOGY AND DISEASE

Role of septins in microbial infection

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ABSTRACT

Septins are widely recognized as a component of the cytoskeleton that is essential for cell division, and new work has shown that septins can recognise cell shape by assembling into filaments on membrane regions that display micrometer-scale curvature (e.g. at the cytokinetic furrow). Moreover, infection biology studies have illuminated important roles for septins in mediating the outcome of host–microbe interactions. In this Review, we discuss a selection of mechanistic insights recently gained from studying three infection paradigms: the rice blast fungus *Magnaporthe oryzae*, the poxvirus family member vaccinia virus and the Gram-negative bacterium *Shigella flexneri*. These studies have respectively discovered that higher-order septin assemblies enable fungal invasion into plant cells, entrap viral particles at the plasma membrane and recognize dividing bacterial cells for delivery to lysosomes. Collectively, these insights illustrate how studying septin biology during microbial infection can provide fundamental advances in both cell and infection biology, and suggest new concepts underlying infection control.

KEY WORDS: Cytoskeleton, *Magnaporthe oryzae*, Membrane curvature, Septin, *Shigella flexneri*, Vaccinia virus

Introduction

The study of how microbial pathogens cause disease has inspired a wide variety of research avenues in both cell and infection biology. Pathogenesis is dependent on the coordination of specific microbial effectors and the response of host cell components during infection. To promote invasion, replication and/or dissemination, many pathogens are known to manipulate host cell components for their own advantage. To counteract infection, host cells possess sophisticated defence mechanisms responsible for the elimination of invasive microbial pathogens (Mostowy and Shenoy, 2015; Randow et al., 2013). These include antimicrobial proteins, pathogen-restrictive compartmentalization and host cell death. Recent studies have discovered that the septin cytoskeleton can play a central role in cell-autonomous immunity. Investigations using cellular and animal models have shown that septins can sense pathogenic microbes and promote host defence mechanisms to eliminate them (Mostowy and Shenoy, 2015; Torraca and Mostowy, 2016). In this Review, we highlight examples of infection by a pathogenic fungus, virus and bacterium to illustrate the breadth of discoveries made from studying septin biology during host–microbe interactions. We first provide an updated overview of septin biology, with a particular emphasis on septin assembly and function. We then describe the new links that have been recently discovered between septins and infection by *Magnaporthe oryzae*, vaccinia virus and *Shigella flexneri*. Finally, we discuss differences in the mode of action of septins during infection and also highlight common concepts (e.g. septin recognition of membrane curvature) and their possible implications.

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Septin biology

Septins are GTP-binding proteins discovered in *Saccharomyces cerevisiae* as being essential for cell division (Hartwell, 1971). Septins are expressed in all eukaryotic cells (except in the case of higher plants), and have been implicated in various cellular processes where they act as scaffolds for protein recruitment and diffusion barriers for subcellular compartmentalization (Mostowy and Cossart, 2012; Saarikangas and Barral, 2011). Despite their importance, septins are a relatively poorly understood component of the cytoskeleton, as compared to actin and microtubules. A more-complete understanding of septin biology will be required to effectively treat human diseases in which septins have been implicated, such as cancer, neurological disorders and infection (Hall and Russell, 2004; Mostowy and Cossart, 2012; Peterson and Petty, 2010).

The number of septin genes across organisms is diverse. For example, the nematode *Caenorhabditis elegans* has two, whereas zebrafish (*Danio rerio*) has at least 18. In the case of humans, there are 13 septins categorized into four groups based on sequence similarity: the SETP2 group (SEPT1, SEPT2, SEPT4 and SEPT5), the SEPT3 group (SEPT3, SEPT9 and SEPT12), the SEPT6 group (SEPT6, SEPT8, SEPT10, SEPT11 and SEPT14), and the SEPT7 group (SEPT7) (Mostowy and Cossart, 2012). Structurally, all septins contain a highly conserved central GTP-binding domain flanked by N- and C-terminal regions of variable length and sequence (Fig. 1A). The C-terminus is predicted to form a coiled-coil domain that participates in septin–protein interactions. A short polybasic region (PBR) between the N-terminus and the GTP-binding domain, which binds to negatively charged phospholipids, including phosphatidylinositol 4,5-bisphosphate (PIP2), is found in most septins and is viewed to be responsible for interactions with membrane. Finally, a highly conserved septin-unique element (SUE), which distinguishes septins from other small GTP-binding proteins, is located near the C-terminus and partly overlaps with the GTP-binding interface where it has been suggested from structural studies to participate in septin polymerization (Sirajuddin et al., 2009; Versele et al., 2004).

Septins interact with other septins via their G- and NC-interfaces (Sirajuddin et al., 2007, 2009); the G-interface comprises the GTP-binding domain, whereas the NC-interface comprises the N- and C-terminal regions, which are brought into close proximity upon folding. In this way, septins assemble to form hetero-oligomeric complexes, non-polar filaments and ring-like structures (Fig. 1B). The first crystal structure of a mammalian septin complex revealed a hetero-hexameric composition arranged as SEPT7–SEPT6–SEPT2–SEPT2–SEPT6–SEPT7 (Sirajuddin et al., 2007). In humans, septins can also assemble into hetero-octamers, which additionally contain a member of the SEPT3 group. For example, it is currently viewed that SEPT9 caps the ends of hetero-hexameric complexes by interacting with SEPT7, resulting in a hetero-

Journal of Cell Science (2019) 132, jcs226266. doi:10.1242/jcs.226266

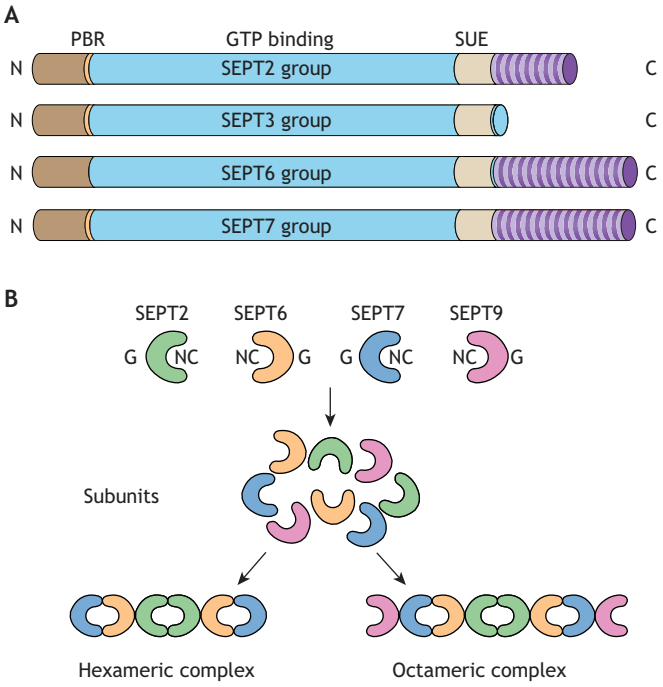

Fig. 1. Human septin architecture and assembly. (A) The domain structure of septin groups. The 13 human septins are categorized into four groups (SEPT2, SEPT3, SEPT6 and SEPT7 groups). All human septins contain a GTP-binding domain and the septin unique element (SUE); most contain a phosphoinositide-binding polybasic region (PBR) except for SEPT6 group members. The length and sequence of the N (proline rich)- and C (coiled-coil)-terminal regions vary. (B) Organization of hetero-hexameric and hetero-octameric septin complexes. Septins from different groups are shown in different colours. Septins interact with other septins via their G- and NC-interfaces to form non-polar complexes; human septins can form complexes that contain three or four septins, where each septin group member is present in two copies, which are symmetrically arranged. Images are adapted with permission from Mostowy and Cossart (2012).

octameric composition arranged as SEPT9-SEPT7-SEPT6-SEPT2-SEPT2-SEPT6-SEPT7-SEPT9. Studies have shown that different members of a group can substitute for one another *in vitro* and *in vivo* (Kinoshita, 2003; Nakahira et al., 2010; Sandrock et al., 2011). Work using RNA interference (RNAi) has shown that SEPT7, the only septin in the SEPT7 group, is important for septin stability (i.e. other septins are unstable and downregulated in its absence) (Estey et al., 2010; Lobato-Márquez et al., 2018; Tooley et al., 2009). From this, it can be concluded that SEPT7 is essential for septin assembly and function in mammals. In agreement with this, the knockout of SEPT7 in mice causes embryonic lethality (Ageta-Ishihara et al., 2013; Menon et al., 2014).

Septins localize at regions of the cell displaying micrometer-scale curvature, including the cytokinetic furrow and the base of cellular protrusions (e.g. cilia and dendritic spines) (Mostowy and Cossart, 2012; Saarikangas and Barral, 2011). Remarkably, work using purified recombinant proteins has shown that septins preferentially recognize micrometer-scale membrane curvature (Bridges et al., 2016). These observations indicate that recognition of curvature is an intrinsic property of the septin cytoskeleton, which enables it to sense the morphology of cells, organelles and local membrane subdomains. Importantly, this feature is different from other sensor proteins (e.g. Bin-Amphiphysin-Rvs domain-containing proteins) that detect curvature at the nanometer scale (McMahon and Boucrot, 2015). Interestingly, new work has shown that an amphipathic helix (AH) is necessary and sufficient for septin curvature sensing both *in vitro* and *in vivo* (Cannon et al., 2019), and that septin filaments can reorganize in response to changes in membrane curvature (Beber et al., 2019). Considering their important roles in health and disease (Hall and Russell, 2004; Mostowy and Cossart, 2012; Peterson and Petty, 2010), how septins assemble and how they work in conjunction with actin, microtubules and phospholipids, are the focus of intense investigation.

Septin rearrangements during microbial infection

There are several examples for pathogenic microbes interacting with septins, and studying infection has made key contributions to the understanding septin biology (Mostowy and Cossart, 2012; Torraca and Mostowy, 2016). In this section, we focus on mechanistic insights recently gained from studying three infection paradigms: the fungus *M. oryzae*, vaccinia virus and the Gram-negative bacterium *S. flexneri*.

Exploitation of septins by *M. oryzae*

The rice blast fungus *M. oryzae* is responsible for disease in rice and other cereal crops, and is recognized as a major threat to global food security (Fernandez and Orth, 2018). *M. oryzae* has also emerged as an important model to study the process of plant infection by pathogenic fungi. *M. oryzae* initiates plant infection when a three-celled conidium adheres to the rice plant surface (Fernandez and Orth, 2018). Following attachment, a germ tube is produced from the conidium to form a dome-shaped structure called the 'appressorium', a specialized infection cell from which a penetration peg develops to rupture the leaf cuticle. After penetration, the peg invades the underlying plant epidermal cells to form a filamentous hypha, which secretes effector proteins to promote fungal infection. Despite important progress, the mechanism of appressorium-mediated plant infection by *M. oryzae* is not fully understood.

Pioneering work led to the discovery that the appressorium mediates host cell penetration using a septin-dependent mechanism (Dagdas et al., 2012). In *M. oryzae*, there are five septins, four of which (Sep3, Sep4, Sep5 and Sep6) show homology to *S. cerevisiae* septins (Cdc3, Cdc10, Cdc11 and Cdc12). Strikingly, confocal microscopy showed that septins colocalize with a filamentous actin (F-actin) network as ∼6.0 µm ring-like structures at the appressorium pore (Fig. 2A), which are ten times the size of septin rings in mammalian cells (Dagdas et al., 2012). Depletion of *SEP3*, *SEP4*, *SEP5* or *SEP6* in *M. oryzae* leads to delocalization of the other remaining septins, suggesting that septins work together to form a large ring, akin to a 'collar'. Organization of the appressorium F-actin network is also disrupted in the absence of septins, indicating that septin ring assembly is required for scaffolding the F-actin network at the base of the appressorium. Septins interact with specific phosphatidylinositols (PtdIns) (e.g. PtdIns-4-phosphate, PIP2) at the appressorium pore, where they scaffold cortical F-actin via the ezrin–radixin–moesin (ERM) protein Tea1 (Dagdas et al., 2012). In addition to the scaffolding function, the septin ring also acts as a diffusion barrier to mediate positioning of proteins required for penetration peg formation, such as Las17 (an Arp2/3 complex nucleation promoting factor implicated in F-actin polymerization) and Rvs167 (a Bin–Amphiphysin–Rvs domain-containing protein implicated in regulating membrane curvature) (Dagdas et al., 2012). Importantly, *M. oryzae* cells with septin mutations showed significantly reduced pathogenesis due to their inability to rupture leaf cuticles, highlighting a requirement for septins in development of rice blast disease (Dagdas et al., 2012).

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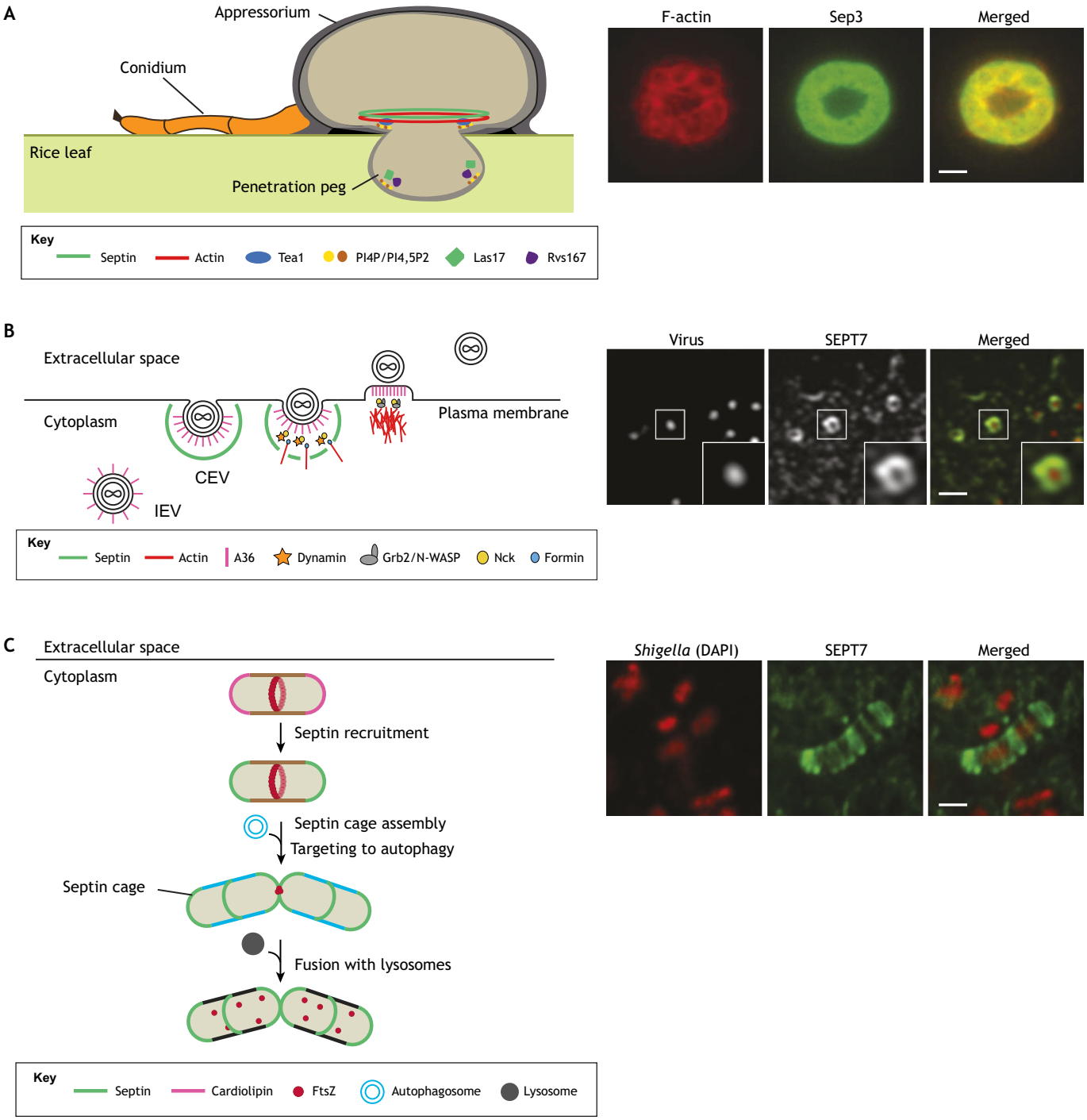

Fig. 2. Septins and microbial infection. (A) Left, cartoon depicting the process of septin-mediated plant infection by *M. oryzae*. A septin ring forms around the appressorium pore where *M. oryzae* develops a penetration peg to breach the leaf surface. Here, septins recruit a toroidal F-actin network through interactions with specific PtdIns [e.g. PI4P, PI(4,5)P_2] and Tea1 to provide cortical rigidity at the plasma membrane. Here, septins can also act as a diffusion barrier to position proteins required for penetration peg formation, such as Las17 and Rvs167. Right, confocal microscopy image of F-actin (red) and septin ring (Sep3, green) assembly at the *M. oryzae* appressorium pore. Scale bar: 10 µm. Images adapted with permission from Dagdas et al. (2012). (B) Left, cartoon depicting the role of septins in the prevention of virus spread. During viral egress, septins are recruited to vaccinia virus immediately after its fusion with the plasma membrane. Here, septins form ring-like structures to entrap extracellular viruses. However, septins are displaced from the virus when it induces actin polymerization. Dynamin (recruited by Nck) and formin-mediated actin polymerization work together to displace septins from the virus. Right, confocal microscopy image of septin entrapment (SEPT7, green) of vaccinia virus (red) at the plasma membrane. Scale bar: 1 µm. Images adapted with permission from Pfanzelter et al. (2018). (C) Left, cartoon depicting *Shigella* septin cage assembly and its bactericial action. Septins are recruited to regions of micrometer-scale membrane curvature presented by dividing *Shigella* cells in the cytoplasm. Here, septin recruitment is promoted by cardiolipin, which is enriched at such regions. Following recruitment, septins assemble into cages around growing *Shigella* cells to inhibit cell division through induction of autophagy and lysosome fusion. Right, confocal microscopy images of septin recruitment (SEPT7, green) to actively dividing *Shigella* (red). Scale bar: 1 µm. Images adapted with permission from Krokowski et al. (2018) where it was published under a Creative Commons Attribution license (CC BY 4.0).

NADPH oxidases have been implicated in various cellular processes in plants, including septin-mediated plant infection by *M. oryzae* (Ryder et al., 2013). F-actin organization at the appressorium pore requires NADPH oxidases (Nox1 and Nox2) and their regulatory subunit NoxR, whereas Nox2 and NoxR are sufficient for septin ring assembly. Consistent with this, the localization of Tea1, Las17 and Rvs167 are disrupted in the absence of Nox2 and NoxR, but not Nox1 (Ryder et al., 2013). These results indicate that ERM–actin interactions at the appressorium pore, as well as septin-mediated localization of Las17 and Rvs167, require the Nox2–NoxR complex.

New work has shown that cell-to-cell invasion by *M. oryzae* is mediated by a fungal mitogen-activated protein kinase (MAPK) called Pmk1 (Sakulkoo et al., 2018). Pharmacological inhibition of Pmk1 prevented *M. oryzae* from invading adjacent plant cells because the fungus becomes trapped within a primary infected cell. In this case, septins accumulate at cell wall contact points but fail to form the septin 'collars' required for the development of penetration hyphae at plasmodesmata (cell–to–cell junctions) (Sakulkoo et al., 2018). SMO1, a GTPase-activating protein involved in regulation of Ras signalling, has been identified as interacting with components of the Pmk1 MAPK signalling pathway and is required for adherence of conidia to plant surface and appressorium development (Kershaw et al., 2018). In line with this, a Δ*smo1* mutant showed disorganization of the septin ring and F-actin network at the appressorium pore, and co-immunoprecipitation experiments revealed Smo1 interaction with septins (Sep3, Sep4, Sep5 and Sep6) (Kershaw et al., 2018). These results show that septin-mediated organization of F-actin is regulated by SMO1.

Together, these studies uncover a novel mechanism by which *M. oryzae* employs septins for the development of a rigid penetration peg to rupture the leaf cuticle and invade the host plant cell (Fig. 2A). It is tempting to speculate that membrane curvature mediates the recruitment and assembly of septins at the appressorium pore, since this point may exhibit sufficient indentation and polarization for this purpose. These studies also suggest new strategies to combat this important fungal pathogen, possibly by targeting fungal septins to prevent plant cell invasion. In the future, it will therefore be interesting to test whether septins also promote the infection of other pathogenic fungi, and to discover additional septin functions in fungal biology. In the case of infection by *Candida albicans*, a human fungal pathogen, our work has shown that SEPT7 is required for the accumulation of the host receptor N-cadherin around *C. albicans* hyphae during endocytosis (Phan et al., 2013).

Septin entrapment of vaccinia virus

Vaccinia virus, a member of the *Orthopoxviruses* genus, is well known for its use as a vaccine to successfully eradicate smallpox (Walsh and Dolin, 2011). Vaccinia is also a valuable tool in cell biology used to study rearrangements of the actin and microtubule cytoskeletons during infection (Leite and Way, 2015). Following host cell invasion, vaccinia replicates in the cytoplasm and is wrapped by two membranes derived from the trans-Golgi network or endosomal cisternae to form intracellular enveloped virus (IEV). IEV is transported to the cell periphery in a microtubule-dependent manner, where the outermost membrane of IEV fuses with the plasma membrane to form cell-associated enveloped virus (CEV) on the outer cell surface (Fig. 2B). CEV-mediated actin tail formation is crucial for virus spread between adjacent cells (Leite and Way, 2015). Vaccinia actin tails are generated by phosphorylation of two specific tyrosine residues, Y112 and Y132 on the viral transmembrane protein A36 by Src and Abl kinases (Leite and Way, 2015). Phosphorylated Y112 recruits the adaptor protein Nck (both Nck1 and Nck2), Wiskott-Aldrich syndrome protein-interacting protein (WIP; also known as WIPF1) and neural Wiskott–Aldrich syndrome protein (N-WASP; also known as WASL) to induce Arp2/3-dependent actin polymerization, while phosphorylated Y132 recruits growth factor receptor-bound protein 2 (Grb2) to help stabilize the Nck, WIP and N-WASP signalling network and enhance actin tail formation (Leite and Way, 2015). Although vaccinia virus egress has been the subject of intense investigation (Welch and Way, 2013), the detailed mechanism of membrane fusion prior to virus release remains unclear.

Two human genome-wide RNAi screens originally suggested that septins can impact on the propagation of vaccinia virus (Beard et al., 2014; Sivan et al., 2013). However, the precise role of septins in vaccinia infection was unknown until recently. Consistent with septins playing a role in viral spreading (Beard et al., 2014; Sivan et al., 2013), our recent work has shown that siRNA-mediated depletion of SEPT7 leads to a significant increase in release of vaccinia virus (Pfanzelter et al., 2018). Further careful analysis revealed that SEPT7 depletion increased the number and length of CEV actin tails, but did not affect their velocity or directionality. Similar results were observed in cells depleted for SEPT2, SEPT9 or SEPT11. These results suggest that septin complexes control vaccinia infection by suppressing actin tail formation, viral release and cell-to-cell spreading. Consistent with this, high-resolution confocal microscopy showed that septins are recruited to viral particles only after their fusion with the plasma membrane, and that SEPT7 forms ring-like structures, thereby entrapping extracellular CEV (Fig. 2B). Strikingly, the number of CEV with SEPT7 recruitment increased to ∼85% in cells that had been infected with A36-YdF virus (a vaccinia strain deficient in actin tail formation, since A36 cannot be phosphorylated on Y112 or Y132) from ∼14% in cells infected with wild-type vaccinia, suggesting that actin polymerization promotes septin displacement from the virus. However, chemical inhibition of the Arp2/3 complex or the use of N-WASP$^{-/-}$ mouse embryonic fibroblasts (MEFs) did not affect the recruitment of SEPT7 to CEV, indicating that Arp2/3-mediated actin tail formation does not displace septins from the virus (Pfanzelter et al., 2018). A possible explanation for this result is that upstream regulators of the actin polymerization machinery (other than the Arp2/3 complex) are responsible for septin displacement.

Interestingly, A36-YdF virus is able to induce activation of Src and Abl family tyrosine kinases, raising the question of whether phosphorylation of A36 promotes loss of septins from the virus. Furthermore, pharmacological inhibition of Src and Abl family kinases caused an increase in the number of wild-type CEV that recruit SEPT7 (to the same level as A36-YdF virus), suggesting that phosphorylation of A36 regulates septin displacement. Indeed, experiments using virus mutants that are deficient in Nck and Grb2 recruitment (A36-Y112F and A36-Y132F, respectively) indicated the involvement of Nck in regulating septins at the CEV. Consistent with this, infection of Nck$^{-/-}$ mouse embryonic fibroblasts (MEFs; lacking both Nck1 and Nck2) showed that SEPT7 recruitment is equally high for both wild-type and A36-YdF virus, demonstrating that Nck recruitment (mediated by phosphorylated Y112 of A36) negatively influences the localization of septins to CEVs. By analysing a series of Nck mutants, it was shown that the third SH3 domain is both necessary and sufficient to displace septins from the virus (Pfanzelter et al., 2018). Furthermore, we could demonstrate that Nck-mediated recruitment of dynamin promotes the loss of septins from CEV prior to actin tail formation. Treatment of HeLa

cells with SMIFH2, a formin inhibitor, increased the number of CEVs co-localizing with septins. By contrast, simultaneous inhibition of dynamin and formin did not increase the number of CEVs with septins as compared to when either dynamin or formin alone was inhibited, suggesting that dynamin and formin-mediated actin polymerization work together to displace septins from CEV (Pfanzelter et al., 2018).

Vaccinia virus has been used for human immunization more than any other vaccine, yet the cell-autonomous immune response to vaccinia is not fully known. Our recent study has revealed, for the first time, that septins suppress vaccinia release by entrapping virus at the plasma membrane, and that the antiviral function of septins is regulated by dynamin and formin-mediated actin polymerization (Fig. 2B). It will be of great interest to identify the formin that displaces septins from CEVs and test its precise relationship with dynamin. Considering the role of septins in membrane curvature recognition, it would be interesting to address whether recruitment of septins to sites where CEV fuses with the plasma membrane depends on membrane curvature. Interestingly, new work has shown that the protease NS2B–NS3 heterodimer from Zika virus, a mosquito-transmitted *Flavivirus* that causes microcephaly, can mediate cytokinesis defects and cell death through cleavage of SEPT2 (Li et al., 2019). The role of septins in infection by other pathogenic viruses has not yet been tested.

Septin interactions with *S. flexneri*

S. flexneri is a member of the Enterobacteriaceae family that causes bacillary dysentery (also called shigellosis). *Shigella* causes ∼165 million illness episodes worldwide and is responsible for ∼164,000 deaths annually (Kotloff et al., 2018). The World Health Organization (WHO) has listed *Shigella* among the top 12 priority pathogens requiring urgent action because of its emerging antibiotic resistance (Tacconelli et al., 2018). *Shigella* is also recognized as an exceptional model organism to study cell biology and cell-autonomous immunity. The infection cycle of *Shigella* has been well studied: bacteria induce their entry into epithelial cells, replicate in the cytoplasm and polymerize actin tails at their surface, which allow bacteria to move in the cytoplasm, evade cell-autonomous immunity and spread into neighbouring cells (Welch and Way, 2013). The *Shigella* invasion process is strictly dependent on its type 3 secretion system (T3SS), a needle-like apparatus that allows delivery of bacterial effector proteins into the target eukaryotic cell (Killackey et al., 2016). To defend against *Shigella* invasion, host cells use a variety of mechanisms to restrict bacterial proliferation and dissemination, including septin-mediated cell-autonomous immunity (Mostowy and Shenoy, 2015; Randow et al., 2013). In the case of septin-mediated cell-autonomous immunity, nearly a decade of research has shown that septins entrap *Shigella* in cage-like structures (Fig. 2C) to prevent their actin-based motility and target bacteria to degradation by autophagy (Mostowy et al., 2010; Sirianni et al., 2016). However, septins and the autophagy machinery can also promote the proliferation of intracellular *Shigella* that are not entrapped in septin cages, possibly by regulating host cell glycolysis (Lobato-Márquez et al., 2018). In that regard, we showed that the proliferation of *Shigella* not entrapped in septin cages is reduced upon depletion of SEPT2 or SEPT7 because host cell glycolysis (a primary energy source consumed by *Shigella* to promote its proliferation) is dysregulated.

How septins recognize bacteria for cage entrapment was unknown until our recent work demonstrated that septins recognize the membrane curvature of dividing bacterial cells to mediate their entrapment and delivery to lysosomes (Krokowski et al., 2018). By using time-lapse microscopy, we showed that for the vast majority (∼87%) of entrapped *Shigella* cells (which are ∼1.0 µm in diameter), SEPT6 is first recruited to the division site and/or cell poles, which both display a high curvature, before complexes containing SEPT6 assemble into cage-like structures (Krokowski et al., 2018). These observations are consistent with previous findings showing that septins recognize micrometer-scale curvature of eukaryotic membrane (Bridges et al., 2016). Remarkably, a variety of high-resolution microscopy techniques has revealed that the localization of SEPT6 overlaps with that of *Shigella* FtsZ, a tubulin-like protein, which forms a contractile ring structure (the Z-ring) at the bacterial division site, suggesting that the curvature generated by Z-ring constriction promotes septin localization to the bacterial division site (Krokowski et al., 2018). Similar results have been observed for a variety of invasive bacteria (and important human pathogens), including *Shigella sonnei*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Krokowski et al., 2018). Furthermore, *in vitro* experiments using purified septins to test for lipid-binding has revealed that septins bind to cardiolipin, an anionic phospholipid enriched at the *Shigella* cell division site and poles. In addition, use of silica beads coated with *Shigella* membrane showed that cardiolipin promotes septin recruitment to beads of 1 µm in size (i.e. the same diameter as *Shigella* cells). Moreover, when bacterial cell division was pharmacologically inhibited at the stage of DNA replication, septins were still recruited to poles of bacterial cells, but failed to assemble into cages, indicating that bacterial cell growth is required for septin cage entrapment.

How does septin caging affect bacterial cell division? Time-lapse microscopy imaging revealed that ∼93% of bacteria fail to divide following their entrapment in a septin cage. Interestingly *Shigella* that recruit both septins and the autophagy machinery [i.e. p62 (also known as SQSTM1; an autophagy receptor) and LC3B (also known as MAP1LC3B)] present significantly less Z-ring-positive bacterial cells (an indication of actively dividing bacterial cells), as compared to *Shigella* that recruit either septins or the autophagy machinery alone. We do not yet understand what distinguishes bacteria that recruit both septins and the autophagy machinery from those that recruit only septins or autophagy factors alone. However, these results suggest that septins are necessary but not sufficient to inhibit bacterial cell division, and that recruitment of the autophagy machinery is also required. Finally, pharmacological inhibition of lysosome acidification resulted in the failure of septin-cage-entrapped *Shigella* to disassemble their Z-ring, strongly suggesting that lysosome fusion is necessary to fully inhibit bacterial cell division. Taken together, these results demonstrate that septin cages inhibit bacterial cell division by autophagy and delivery to lysosomes (Krokowski et al., 2018) (Fig. 2C). Although, the precise role of septins in autophagy is not fully clear, a recent study using budding yeast has suggested a role for septins during autophagosome formation (Barve et al., 2018).

Shigella remains a global health threat and thus a more complete understanding of host factors that control *Shigella* infection is required. Although investigation of septin cage formation has revealed a fundamental mechanism used by host cells to recognize and destroy intracellular bacterial pathogens, more work is necessary to determine how septin biology could be used to control *Shigella* and other antibiotic resistant infections. We also suggest that understanding the bacterial cytoskeleton during host–pathogen interactions can inspire development of new therapeutic regimes for infection control (Krokowski et al., 2019). Considering our discovery that the bacterial actin homologue MreB can promote actin tail formation (Krokowski et al., 2019), MreB can also be viewed as a promising target for antimicrobials. This reported biology is proposing

Box 1. Remaining questions in the field of septin–microbe interactions

- In the case of *Magnaporthe* infection, why are septin ring-like structures at the appressorium pore ten times larger than septin rings observed in mammalian cells? Can septin biology and recognition of membrane curvature guide the development of anti-fungals?
- In the case of vaccinia virus, how are septins recruited to sites where the CEV fuses with the plasma membrane? Does it depend on micrometer-scale membrane curvature and a septin AH?
- In the case of *Shigella*, what bacterial factors (in addition to membrane curvature and cardiolipin) contribute to septin cage recruitment and/or assembly? Are there bacterial effectors that impair septin caging?
- What is the role of septins in patients infected with microbial pathogens? It is important to study the interplay between septins and microbial pathogens by looking at cells isolated from infected patients. Can we use septins to combat infection and antimicrobial resistance?

a novel approach to future medicine and should encourage further work to exploit the cytoskeleton to treat bacterial infection.

Conclusions and future perspectives

Collectively, the studies highlighted in this Review reveal that septins act as structural determinants that are important to control infection by diverse microbial pathogens. These studies prompt several outstanding questions and inspire a wide variety of exciting research avenues (Box 1). In the case of *M. oryzae*, this fungal pathogen forms a large septin ring at the appressorium pore to promote the development of a penetration peg needed for its plant cell invasion. In addition, work with vaccinia virus and *S. flexneri* have revealed a crucial role for host cell septins in entrapping pathogens to prevent their dissemination. Considering that septins can recognize the membrane curvature of dividing *Shigella*, membrane curvature can be viewed as a fundamental 'danger signal' during microbial infection. It is thus of great of interest to study the wider role of membrane curvature in microbial pathogenesis and septin-mediated host immunity. Moreover, it will be important to understand the full breadth of microbial pathogens that are regulated by septin biology. In the case of bacteria, work has shown that septins are recruited to sites of invasion for several pathogens (Boddy et al., 2018; Kühbacher et al., 2015; Mostowy et al., 2009a,b, 2011) and accumulate at sites of *Clostridium difficile* protrusion initiation (Nölke et al., 2016), as well as entrapping cytoplasmic enteropathogenic *Escherichia coli* (EPEC) in cage-like structures (Lee et al., 2017). Another key question is to understand the multiple roles for septins in host defence *in vivo*. In this regard, zebrafish larvae have been a useful *in vivo* model to study septin-mediated immunity (Duggan and Mostowy, 2018). We propose that a better understanding of septins during microbial infection can help to inspire new strategies aimed at infection control and possibly other autoimmune or inflammatory diseases that implicate septins.

Acknowledgements

We thank Tolga Bozkurt, Julia Pfanzelter and Sina Krokowski for helpful discussions and manuscript feedback.

Competing interests

The authors declare no competing or financial interests.

Funding

H.V.N. is funded by a Newton International Fellowship from the Royal Society (NIF\R1\180035). Work in the S.M. laboratory is supported by a Wellcome Trust Senior Research Fellowship (206444/Z/17/Z) and the Lister Institute of Preventive Medicine.

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