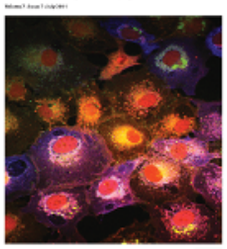
Autophagy and the cytoskeleton New links revealed by intracellular pathogens

Serge Mostowy, Pascale Cossart

Autophagy



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Autophagy and the cytoskeleton

New links revealed by intracellular pathogens

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Actin-based motility is used by various pathogens such as *Listeria* and *Shigella* for dissemination within cells and tissues, yet host factors counteracting this process have not been identified. We have recently discovered that infected host cells can prevent actin-based motility of *Shigella* by compartmentalizing bacteria inside 'septin cages,' revealing a novel mechanism of host defense that restricts dissemination. Because bacterial proteins controlling actin-based motility also regulate the autophagy process, we hypothesized and then established a link between septin caging and autophagy. Together, these results unveiled the first cellular mechanism that counteracts pathogen dissemination. Understanding the role of septins, a so far poorly characterized component of the cytoskeleton, will thus provide new insights into bacterial infection and autophagy.

Key words: actin, autophagy, *Listeria*, *Shigella*, septin

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Several intracellular pathogens have developed strategies to avoid destruction by cytosolic immune responses. A well-studied strategy for efficient infection, employed by different pathogens, including *Listeria monocytogenes* and *Shigella flexneri*, is the subversion of the actin cytoskeleton to move intra- and intercellularly. The proteins required for *Listeria* and *Shigella* actin-based motility have been well characterized. *Listeria* ActA directly recruits and activates the Arp2/3 complex to polymerize actin and generate actin tails. *Shigella* IcsA recruits N-WASP, which then recruits and activates the Arp2/3 complex. Recent work has revealed that both ActA and IcsA also play key roles in the autophagic escape of *Listeria* and *Shigella*. ActA protects *Listeria* from ubiquitination and p62 recruitment, thereby preventing recognition of *Listeria* by autophagy. *Shigella*'s escape from autophagy is mediated by IcsB, a bacterial effector protein, which interacts with IcsA and prevents the interaction of IcsA with the autophagy protein Atg5. Emerging from these studies is the concept that proteins involved in actin-based motility are also connected to the autophagy process.

Septins are a relatively newly characterized component of the cell cytoskeleton. Discovered in *Saccharomyces cerevisiae* where they organize as a ring at the bud neck, septins are GTP-binding proteins gaining increasing recognition as key players in the regulation of cell division, cytoskeletal dynamics and membrane remodeling. Unlike actin and microtubules, septins assemble into nonpolar filaments and are regarded as unconventional cytoskeletal components because they associate with cellular membranes, actin filaments and microtubules. Yet, how septins function as a distinct component of the cytoskeleton remains to be determined. Solving this issue is a challenge given the high number of septin isoforms (e.g., 14 in humans) and their varying distribution in different cell types. Complicating the situation further, different septins form polymers with the smallest functional unit of human septins being a hexamer.

Despite the well-established picture of proteins and mechanisms regulating actin-based motility, septin function had not been addressed. We have recently shown that septin assemblies are recruited to different bacteria that polymerize actin in the

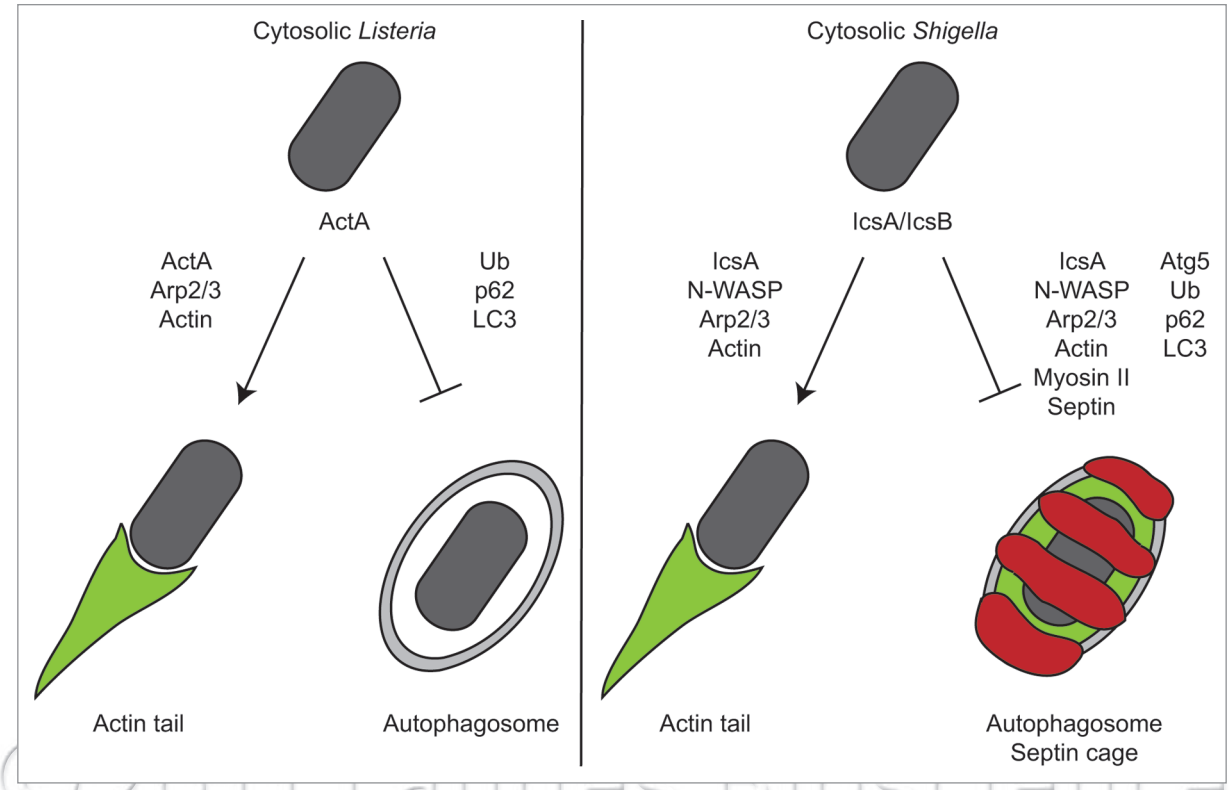

Figure 1. Intracytosolic lifestyles of *Listeria* and *Shigella*. Left part. *Listeria* ActA allows Arp2/3 recruitment and actin tail formation. It also prevents ubiquitinated protein recruitment/formation (Ub), p62 recognition and LC3 recruitment. Right part. *Shigella* IcsA recruits N-WASP and allows Arp2/3 recruitment and actin tail formation. *Shigella* IcsB binding to IcsA may protect against Atg5 interaction and IcsA-mediated actin polymerization is required for ubiquitinated protein recruitment/formation, p62 recognition, LC3 recruitment and septin caging.

cytosol of infected cells. Strikingly, intracytosolic *Shigella* either become compartmentalized in septin cage-like structures or form actin tails, suggesting an inverse relationship between septin caging and actin tail formation. Inhibition of actin polymerization inhibits cage formation, revealing that actin polymerization is critical to both tail formation and septin caging. Moreover, inactivation of septin caging by siRNA against septin or by inhibiting myosin II function using siRNA or blebbistatin to reduce the affinity of myosin II for actin, increases the number of *Shigella* with actin tails. In contrast, treatment of cells with TNFα, a pleiotropic cytokine known to orchestrate a wide range of biological functions and produced upon *Shigella* infection, stimulates septin caging and restricts actin tail formation and cell-to-cell spread. Together, these results show that septin caging serves to counteract actin-based motility and restrict the dissemination of invasive pathogens.

As *Shigella*-induced actin polymerization and autophagosome formation both depend on IcsA, we reasoned that bacteria in septin cages may be compartmentalized for autophagy. In line with this hypothesis, we showed that autophagy markers (e.g., p62 and GFP-LC3) are recruited to septin cages. As *Shigella* can escape autophagy by a mechanism dependent on IcsB, we analyzed the behavior of an IcsB mutant. *Shigella* lacking IcsB was compartmentalized in septin cages more efficiently than the wild-type strain, confirming that autophagy and septin cage assembly contribute to the same process. Strikingly, when either one of the two septins essential for filament formation (SEPT2 or SEPT9) or critical autophagy components (p62, Atg5, Atg6 or Atg7) were depleted by siRNA, both septin cages and autophagy markers failed to accumulate around *Shigella*. These results highlight an interdependence between the two processes of septin assembly and autophagy. Thus, the mechanisms underlying septin recruitment at the site of autophagosome formation, and more generally how septin assembly is orchestrated with actin polymerization and autophagic membrane recruitment, require further investigation.

Listeria and *Shigella* use similar cellular mechanisms during infection, including actin-based motility and autophagy escape. However, in the case of *Listeria*, no efficient septin caging has been observed. *Listeria* avoids autophagic recognition by expressing ActA and ActA mutants are efficiently targeted by autophagy. Considering that, in the case of *Shigella*, septin cage assembly is both actin dependent and autophagy connected, *Listeria* therefore evades both septin caging and autophagy via its surface expression of ActA. Given these fundamental differences between *Listeria* and *Shigella* (**Fig. 1**), it is clear that in-depth investigation of these two bacteria will help to precisely describe the coordination between actin, septin and selective autophagy.

In summary, we have identified septin caging as a mechanism of host defense that controls the fate of intracytosolic bacteria towards intercellular spread or autophagy. To fully understand its role

in host defense, septin-mediated antibacterial autophagy will have to be studied in vivo across a panel of pathogens. It is likely that other autophagic activities will also benefit from septin assembly, and we have observed, using siRNA, that the cellular levels of autophagy markers p62 and LC3-II are strikingly reduced upon septin depletion. These results suggest a general role for septins in autophagic activity and, together with the concept of septin caging during bacterial infection, provide a so-far-unsuspected view on how the cytosketon is involved in autophagy. A more comprehensive understanding of septin biology should thus provide new insights into how the cytoskeleton can function in autophagy.