

A pathogen's journey in the host cell

Bridges between actin and traffic

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Manipulation of the actin cytoskeleton is a commonly used process by which bacterial pathogens and viruses are able to neutralize host defense mechanisms and subvert them in order to replicate in a hostile environment. Diverse bacteria display a wide array of mechanisms of regulation of microfilaments to enter, move within or exit the host cell. A less studied subject is how pathogens may co-opt the actin cytoskeleton to disturb vesicle trafficking pathways, namely phagolysosomal fusion, and avoid degradation. In fact, although actin plays a role in endosomal trafficking and phagosome maturation, the knowledge on the exact mechanisms and additional players is still scarce. Recently, we found that the *Legionella pneumophila* virulence factor VipA is an actin nucleator, associates with actin filaments and early endosomes during infection, and interferes in yeast organelle trafficking pathways, suggesting it may be linking actin dynamics to endosome biogenesis. Further studies on this protein, together with work on other bacterial effectors, may help shed light in the role of actin in endosomal maturation.

Introduction

Over long processes of coevolution, microbial pathogens have developed a multitude of mechanisms to survive and replicate in the host organism. They have not only neutralized host cell pathways that would ultimately lead to their killing, but have also hijacked eukaryotic networks for their own benefit. Depending on the pathogen

displaying or not an obligatory intracellular phase during the life cycle, these mechanisms may yield responses as diverse and contrasting as promotion or prevention of fundamental eukaryotic processes, such as phagocytosis, apoptosis, autophagy or vesicle trafficking. In order to modulate these pathways, bacterial pathogens inject into the host cell virulence factors, or effector proteins, through secretion systems. These effectors act by targeting specific eukaryotic components, regulating their subcellular localization and/or activity.

Legionella pneumophila Effectors: The Legion of Doom

Legionella pneumophila is a facultative intracellular bacterium that is able to invade and replicate inside phagocytic cells. In its natural habitat, aquatic environments, it parasitizes a wide and diversified group of amoebae. It is believed that the large repertoire of virulence mechanisms acquired during coevolution with protozoa has made *L. pneumophila* competent for infection of human lung macrophages, leading to a frequently fatal type of pneumonia known as Legionnaires' disease. Legionellae thrive in a remodeled compartment inside the host cell, the Legionella-containing vacuole (LCV). Immediately after uptake, the LCV avoids the endocytic pathway and sequentially recruits vesicles trafficking between the ER and the Golgi, mitochondria and ribosomes, becoming a rough ER-like compartment. In this modified niche the bacteria divide until they finally exit the host cell, either by lytic or non-lytic egress mechanisms. Fundamental to these

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processes is a Type IV-B secretion system, called Icm/Dot, which translocates over 300 effectors into the host cell.¹ A particular feature of the *Legionella* effector repertoire is its functional redundancy, which derives from the absence of infection-related phenotypes when one or more of the effector encoding genes are deleted, and therefore complicates the assessment of their role during infection. Nevertheless, during recent years functions have been assigned to a number of them in targeting host cell processes such as apoptosis, the NF κ B pathway or the secretory pathway (for more detailed reviews on *Legionella* effector function please consult^{1,2,3}).

Pathogen Invasion and Actin as a (Cyto)skeleton Key

As actin is one of the most abundant eukaryotic proteins and used in a great variety of structural and regulatory functions in the cell, it is not surprising that the actin cytoskeleton is a major target of pathogenic bacteria and viruses. Co-opting actin dynamics is fundamental in diverse steps of the infection cycle, both for extracellular and intracellular pathogens. Phagocytosis by immune system cells is the first defense mechanism triggered by the host to fight a pathogen. Some extracellular bacteria such as *Yersinia* use effectors to cripple the actin machinery, thus avoiding phagocytosis and consequent degradation, while *E. coli* pathogenic strains hijack the actin cytoskeleton to form an actin pedestal in the site of adhesion, fundamental to the infection cycle, and *Vibrio* promotes microfilament rearrangements disrupting cellular tight junctions in order to disturb the epithelial barrier. In contrast, some intracellular pathogens, namely *Salmonella*, *Shigella* and *Listeria*, stimulate actin rearrangements to promote their own engulfment into otherwise non-phagocytic cells. Once inside the cell, pathogens like *Listeria* or *Rickettsia* evade the phagosome and are able to move within the host cell cytosol and spread to neighboring cells, by triggering the formation of actin comet tails in one pole of the bacterial cell. Vacuolar pathogens, on the other hand, have to counteract the deleterious effects of

phagolysosomal fusion and develop a niche viable for replication. However, although actin has been shown to play a role in processes such as the biogenesis of the Chlamydia inclusion or in enhancing the translocation of type III secretion substrates, relatively less is known about its importance in the establishment of a replicative niche that allows for microbe replication (for references and pathogen subversion of other cytoskeleton components please consult recent comprehensive reviews on this topic by⁴⁻⁶).

Actin cytoskeleton dynamics also play a fundamental role in *Legionella* infection. Actin remodeling is essential for phagocytic uptake and several actin-binding proteins have been implicated in the entry process, such as coronin, villidin, α -actinin and filamin.⁷ Analysis of the composition of the LCV during infection of *D. discoideum* has identified, in addition to actin, many other actin related proteins at later times post infection such as coronin, cofilin, myosin II and profilin, Arp2/3 components, actin bundling and capping proteins.^{8,9} However, the function of all these actin related proteins on the LCV membrane and the cross-talk between *L. pneumophila* effectors and the host actin cytoskeleton components has remained for the most part elusive.

Bridging Actin and the Endosomal Pathway

In our recent paper,¹⁰ we found that the *L. pneumophila* effector VipA binds and nucleates actin polymerization in vitro. During infection of macrophages, translocated VipA co-localizes with actin filaments and early endosomes, an association also observed when VipA-GFP was ectopically expressed in *S. cerevisiae* and in mammalian CHO cells. In previous work,¹¹ it had been shown that this effector impaired vacuolar protein trafficking in *S. cerevisiae*, interfering with the Multivesicular Body (MVB) pathway, which is supported by its colocalization with some MVB components. In addition, we isolated a VipA mutant carrying a mutation in the N-terminal region of the protein (VipA-1), which is affected in its binding and polymerization of actin, in its ability to interfere with the MVB

pathway and subcellular localization. Taken together, these results suggest a role for VipA as a link between the host actin cytoskeleton and endosomal trafficking, a novel function among prokaryotic virulence factors.

To date, only a small number of bacterial actin nucleators have been identified and characterized (Fig. 1). Chlamydia Tarp and *Salmonella* SipC are involved in actin-mediated uptake of the pathogen, while *Rickettsia* Sca2 is a formin-like nucleator that mediates intracellular movement of the bacterium via polymerization of actin comet tails.¹²⁻¹⁴ In addition, the extracellular bacterium *Vibrio parahaemolyticus* injects into host epithelial cells VopL and VopF, which lead to a rearrangement of the actin network and cause disruption of cell-cell contacts, facilitating enteric infection.^{15,16} None of these bacterial actin nucleators have however been implicated in vesicle trafficking, and to our knowledge only two effectors have given clues as to how the pathogen may link actin dynamics to endosomal trafficking for their own benefit. The *Salmonella enterica* phosphoinositide phosphatase SopB plays multiple roles during infection. In addition to mediating actin-dependent internalization of the bacteria, it also prevents the transition of PI(3)P to PI(3,5)P in the *Salmonella* containing vacuole (SCV) membrane and therefore may contribute to arresting SCV maturation along the endosomal pathway and consequently lysosomal fusion.¹⁷⁻¹⁹ Enteropathogenic *Escherichia coli* (EPEC) EspF nucleates a multiprotein complex containing the Arp2/3 activator N-WASP and the endocytic regulator sorting nexin 9 (SNX9), which mediates clathrin-dependent endocytosis. Although the mechanism or pathogenic significance of this has not been yet elucidated, EspF has been proposed to alter SNX9 regulation of endocytosis during EPEC infection, leading to the formation of aberrant tubular vesicles.²⁰⁻²²

Clues From Eukaryotes

Even among eukaryotic proteins only a few have been reported to be simultaneously involved in actin dynamics and endosomal trafficking. Alix (also known as

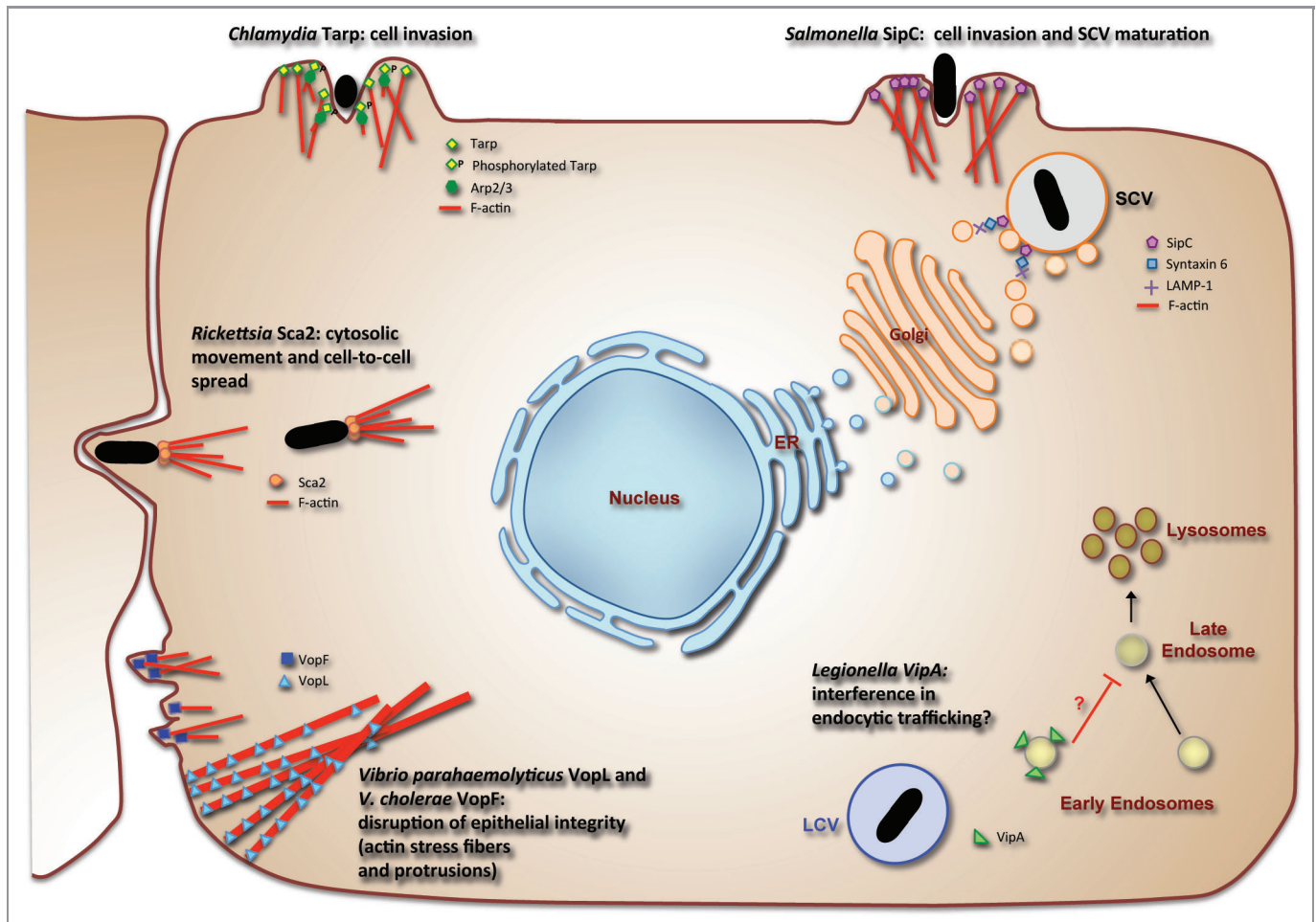


Figure 1. Bacterial actin nucleators and their role in infection. Schematic diagram of cell rearrangements led by bacterial effector-driven actin polymerization. In *Chlamydia*, a cooperative mechanism leading to the formation of cell protrusions and invasion has been proposed, in which Tarp nucleates short actin filaments and when phosphorylated also leads to the recruitment of Arp2/3 via Rac signaling.^{12,30} *Salmonella* SipC promotes entry by direct microfilament assembly and, in addition, is involved in the recruitment of Golgi-derived vesicles with the SCV, by associating with host Syntaxin-6, which in turn binds lysosomal LAMP-1^{13,31}. The formin-like Sca2 nucleator from *Rickettsia* leads to the formation of actin comet tails, which enable the movement of the pathogen within the host cell cytosol, as well as invasion of neighboring cells.¹⁴ *Vibrio cholerae* VopF and *Vibrio parahaemolyticus* VopL (and ortholog VopN from *V. cholerae*) lead to membrane protrusions and formation of stress fibers, respectively, implicated in disruption of tight junctions and consequent epithelial integrity.^{15,16,32} In addition to nucleating actin filaments, *L. pneumophila* VipA binds early endosomes and may play a role in the endocytic pathway.¹⁰

Aip1) is an adaptor protein with multiple functions and binding partners. It associates with actin, tubulins, focal adhesion kinase (FAK), Src and proline-rich tyrosine kinase 2 (PYK-2). It is involved in the actin-dependent positioning of endosomes by connecting these organelles to the cortical actin cytoskeleton.^{23,24} In addition, it interacts with ESCRT components, being involved in the MVB Pathway and thus playing a role in related processes such as EGFR downregulation, HIV budding and also cytokinesis.^{25,26}

Annexins have also been implicated in a dual function as modulators of endosomal trafficking and actin dynamics. Annexin

A6 is a Ca^{2+} -dependent protein that binds lipid rafts in the plasma membrane and endosomes. It also binds F-actin, presumably acting as a scaffold link between membrane microdomains and the cytoskeleton, and regulating transient membrane-actin interactions during endocytic and exocytic transport.^{27,28} The related protein annexin A2 plays a role in early to late endosome transport. Together with Spire1 and Arp2/3, it is involved in MVB formation, by nucleating actin patches in early endosomes that are responsible for the actin-dependent endosome biogenesis, presumably by driving the membrane remodeling process.²⁹

Recent insights into actin regulation of endosome biogenesis came from studies on the Arp2/3 activators WASH and N-WASP. Both play a role in endocytic trafficking, although their functions seem to be temporally and spatially distinct. WASH is endosome-specific (locating mainly in early and recycling endosomes) and it was implicated in actin-regulated endosome shape and scission via Arp2/3 activation.²⁶ In addition, analysis of the trafficking of different receptors (of transferrin, EGF and the acid hydrolase CI-MPR) suggest that membrane remodeling during endosome biogenesis is controlled by WASH-mediated actin

assembly. In contrast, N-WASP appears to function in previous and subsequent endocytic events, by promoting the internalization of plasma membrane patches and later actin-mediated vesicle propulsion through the cytoplasm (reviewed in ref. 4).

Future Perspectives

The localization of VipA to endosomes and its effect on MVB trafficking suggest a role in endosome biogenesis. In the context of a *Legionella* infection, a possible

goal would be a modulation of the interaction of the LCV with the endocytic machinery, thus aiding in preventing its delivery and degradation in the lysosome. Alternative functions related to the MVB pathway could be a disturbance in the adaptive immune response, by interfering with antigen presentation, or in the regulation of membrane lipid and protein composition. In the past, many eukaryotic cell pathways and protein functions have been elucidated by studies on microbe-host interactions. The role played by actin in the endocytic pathway,

particularly in the formation of the MVB, is still scarce and insights may come from further studies on this bacterial effector.

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