

Polymerizer-Mediated Intracellular Movement

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Abstract

Bacterial movement inside the cytoplasm is a major virulence factor in that it is necessary for efficient colonization of the infected tissues. Molecules from both the host and the pathogen present possible sites of pharmacologic intervention. Because locomoting *Listeria* and *Shigella* mimic the activated state of the leading edge of nonmuscle cells, these pathogens are powerful tools for dissecting the molecular machinery of actin-based motility. Analysis of the movement linked to cytoskeleton may lead to: (I) improved understanding of the mechanisms of disease transmission, including carriers and carrier states, pathogen movements, environmental factors and pharmacokinetics of the uptake and residues of vaccines and other biologics, and drugs in cultivated organisms; (II) new therapeutic developments, since it identifies the molecular targets involved in the pathogenicity of *Listeria* and *Shigella* and vaccinia intracellular enveloped virus. Recent knowledge about the intracellular movement in cytoplasm may lead to a better understanding of the processes governing actin dynamics within the cell and disease spread.

Key words: intracellular pathogens, parasite spread, movement in cytoplasm

Intracellular movement

Many microorganisms can exist in an intracellular infectious phase that protects them from the effector functions of the antibody system. These agents infect host cells and live enclosed by the host membrane, in the endosome or free in cytoplasm. Movement is necessary for these parasites to spread within the host animal, it is necessary for their ability to enter host cells, escape from the host cell, and find a new cell. Intracellular pathogens are well known to co-opt cellular machinery to accomplish their life cycle. Many intracellular parasites have special, genetically-encoded mechanisms to get into host cells that are nonphagocytic. Intracellular pathogens such as *Yersinia*, *Listeria*, *Salmonella*, *Shigella* and *Legionella* possess a complex machinery for cellular invasion and intracellular survival. These systems involve various types of virulence factors.

Intracellular parasites have evolved diverse mechanisms to enhance their survival and replication within eukaryotic host cells (Moulder, 1985). These mechanisms largely involve adaptations for survival in distinct intracellular compartments that permit the parasites to avoid lysosomal killing. Parasites can infect different host cells, and once inside the cell, some of them escape from the endocytic vacuole as soon as within an hour of infection.

The interactions between bacterial intracellular parasites and host cells may be divided conceptually into three stages. The first interaction to be considered results in the adherence of bacteria on the surface of the cells. The adherence of invading bacteria is a first major step in the pathogenic process. The second stage of bacteria/host interaction consists of signal transduction events that result in cytoskeletal rearrangements (Tang *et al.*, 1996, Tang *et al.*, 1998) and the third stage is bacterial internalization and intracellular movement (Bliska and Falkow, 1993).

Polymerizer-mediated movement

L. monocytogenes is an intracellular pathogen that survives by passing from phagosomes into cytoplasm. *Listeria* can dramatically stimulate host-cell actin assembly in a directional manner, which serves to rapidly propel the bacteria through the cytoplasm, allowing the organisms to move to peripheral membranes and

spread to uninfected cells. It secretes a hemolytic protein, listeriolysin O (LLO), which mediates bacterial passage into cytoplasm. Measurements of phagosomal pH during infection with *L. monocytogenes* showed that LLO-mediated perforation of phagosomes occurs optimally at pH 6.0, and requires an acidic environment for perforation. It has been shown that only a small percentage of an *L. monocytogenes* population that enters macrophage cells undergoes intracellular growth, while the rest of the bacterial population is killed (Chastellier and Berche, 1994; Raybourne and Bunning, 1994). Chastellier and Berche showed that intracellular multiplication in fact resulted from rapid evasion of a very small number of bacteria (14%) from the phagosome compartment during this early stage to prior phagosome-lysosome fusion (Chastellier and Berche, 1994). By treating cells with DAMP, a weak base that accumulates in the acidic compartment of the cell the authors showed that all phagosomes, whether they contained intact or damaged bacteria, were acidified. Acidification seems to be required for bacterial escape from the phagosome compartment. Listeriolysin is optimally active at an acidified pH (Portnoy *et al.*, 1992).

The intracellular actin-based movement was reported for *L. monocytogenes* by Tilney and Portnoy (1989). The bacteria use proteins inside the host cell to form rocketlike tails. They then ride these tails to the cell wall, deform the wall and invade neighboring cells. *L. monocytogenes* induce polarized actin assembly at the surface to gain propulsive force in infected cells. Upon entering the host cell's cytoplasm, the pathogen *L. monocytogenes* can subvert the normal contractile system of the host cell; subsequent assembly of polar actin-filament structures is likely to provide the force for rapid intracellular bacterial movement and its cell-to-cell spread. The *Listeria* surface protein ActA, which is accumulated over the posterior bacterial body during movement in host cells, is crucial for actin-based motility. The growth of barbed ends, restricted to the bacterial surface is initiated by the bacterial protein ActA (Domann *et al.*, 1992; Kocks *et al.*, 1992; Kocks *et al.*, 1993). Directed *Listeria* movement occurs when a bacterial surface protein, ActA, and cytoplasmic actin-binding proteins distribute to one pole of the bacterium.

The first step in actin filament formation is called nucleation. In this low probability event, three actin monomers are thought to combine simultaneously, forming a thermodynamically unstable trimeric nucleus. Once a trimer is formed, the nucleus most frequently dissociates back into monomers; however, the nucleus occasionally survives long enough to permit subsequent binding of additional actin molecules. Monomer addition is more rapid at the plus end. Once actin filaments reach a steady-state length, ADP-actin monomers are released from the minus ends of the filaments at the same rate as new ATP-actin monomers are added to the plus ends. In the cell, two additional issues are important: (a) actin monomer addition occurs mostly, if not exclusively, at the plus-end; and (b) this process probably produces a pool of ADP-actin from which actin-ATP must be regenerated by exchange (not direct phosphoryl transfer) with ATP in the cytoplasm. ATP-actin has a much higher affinity for the ends of actin filaments than does ADP-actin, and ATP-actin is the primary monomeric species of actin that adds to filament ends in the cell.

The Arp2/3 complex from the cytoplasm, using ActA as a scaffold, constitutes the minimal requirement for the nucleation of actin polymerization, which continues at the interface between the quickly growing (barbed) actin end and the bacterial surface. Insertion of subunits at the barbed end of cross-linked filaments generates compression forces used for propulsion. Within the host cell, actin filament assembly is exquisitely well regulated through the action of a number of actin-regulatory proteins, including actin filament capping and severing proteins, actin monomer sequestering proteins, actin bundling proteins and actin cross-linking proteins. One host cell component likely to play a central role in *Listeria* actin-based motility is profilin. This protein binds to actin monomers in a one to one complex, alters the conformation of the actin monomer, and accelerates the exchange of ATP with actin-bound ADP. Because ATP-actin has a higher affinity for the ends of actin filaments, catalysis of nucleotide exchange should enhance actin-filament assembly. Profilin is likely to be most highly concentrated wherever new actin filaments assemble. Profilin is the only actin regulatory protein that binds polyproline, and the host cell protein vasodilator-stimulated phosphoprotein (VASP) has taken advantage of this unique characteristic to concentrate profilin in specific regions of the cell where new actin filaments assemble. The VASP monomer contains four potential profilin binding sites each containing a series of proline residues. VASP exists as a tetramer in the host cell; therefore each VASP molecule could attract up to 16 profilin molecules. Finally, a third actin-regulatory protein of importance in *Listeria* intracellular movement is called alpha-actinin. This host cell cytoskeletal protein binds to the sides of actin filaments, linking them into bundles. Bundling of actin filaments creates the more rigid co-linear filament network required to form structures such as actin stress fibers and *Listeria* actin tails. The growing actin filaments become crosslinked into the now familiar "comet tail", which acts to anchor the structure so that addition of more actin monomers to the filaments at the gap between the bacterium and the filaments now pushes the bacterium forward. ActA has multiple functional domains and

interacts with several host factors, the Arp2/3 complex, Drosophila Enabled (Ena)/VASP family proteins and PtdIns(4,5)P₂ (Chakraborty *et al.*, 1995; Chakraborty, 1999; Gertler *et al.*, 1996, Welch *et al.*, 1997). The N-terminal domain of ActA (residues 30 to 263) can not only interact with the Arp2/3 complex but can also stimulate its actin nucleation activity (Pistor *et al.*, 2000; Zalevsky *et al.*, 2000). The scientists found that *Listeria* do not move continuously on the molecular level, but instead move in a steplike fashion. Kuo and McGrath (2000) used high-resolution laser tracking to follow the trailing ends of *Listeria* moving in the lamellae of COS7 cells, and found that pauses during motility occur frequently and that episodes of step-like motion often show pauses spaced at about 5.4 nm. Each step may correspond to the addition of individual protein building blocks to the tail. Surprising information about *Listeria* movement has been provided by Buchwalow *et al.* (1997) who reported that the comet tails involved in *Listeria* movement have a tubulin-like component.

Shigella causes bacillary dysentery, a disease provoking severe bloody and mucous diarrhea. When the pathogen reaches the colon, bacteria translocate through the epithelial barrier by way of the M cells that overlay the solitary lymphoid nodules (Sansone *et al.*, 1991; Sansone *et al.*, 1996). The movement of intracellular *Shigella* was first reported by Ogawa *et al.* (1968). The *virG* (also called *icsA*) gene on the large plasmid of *S. flexneri* is required for cell-to-cell spreading and actin-based motility in mammalian cells (Makino *et al.*, 1986; Bernardini *et al.*, 1989). *Shigella* internalization involves cytoskeletal rearrangements that lead to dramatic changes in the plasma membrane at the point of direct contact between the bacterium and the host cell. To propel itself in infected cells, the pathogen *S. flexneri* subverts the Cdc42-controlled machinery responsible for actin assembly during filopodia formation. Bacterial protein IcsA binds N-WASP and activates it in a Cdc42-like fashion. Dramatic stimulation of actin assembly is linked to the formation of a ternary IcsA-N-WASP-Arp2/3 complex, which nucleates actin polymerization. VASP is not involved in *Shigella* movement, and the function of profilin does not require its binding to proline-rich regions. This process promotes a random intracellular movement of the bacteria and leads to the infection of adjacent cells by the formation of protrusions. This movement, which involves the nucleation, polymerization, and subsequent polarization of actin, is referred to as the Ics phenotype (intra/intercellular spread) (Egile *et al.*, 1999).

Spotted fever group *Rickettsia*, and the vaccinia virus also induce polarized actin assembly at the surface to gain propulsive force in infected cells. The viral particles are too large to move from their replication site near the center of the cell to the cell periphery by passive diffusion. The enveloped form of vaccinia virus, called intracellular enveloped virus (IEV), also induces formation of an actin comet tail in infected cells (Cudmore *et al.*, 1995). Unlike *Listeria*, vaccinia forms actin comet tails only at the periphery of infected cells, where the virus can interact with the plasma membrane. The mechanism of the actin tail formation of IEV resembles that of *Shigella* VirG more than that of *Listeria* ActA with respect to the involvement of N-WASP. However, vaccinia virus movement occurs depending on protein tyrosine phosphorylation of one of the surface proteins, called A36R (Frischknecht *et al.*, 1999). The tyrosine-phosphorylated A36R links to N-WASP but does so indirectly via binding to adapter proteins such as Nck and WIP. Unlike *Shigella* actin-based motility, the activation of N-WASP is independent of Cdc42 (Moreau *et al.*, 2000). *R. rickettsii* actin filament tails are more stable than those induced by *L. monocytogenes* and contain some of the cellular cytoskeletal-associated components found in the comet tails of *Listeria* and *Shigella*, but not Arp2:3 and N-WASP (Heinzen *et al.*, 1999; Gouin *et al.*, 1999). These structural and compositional differences suggest that the mechanism of actin recruitment and polymerization by *Rickettsia* is unique compared with *Shigella* and *Listeria*.

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