The Protein Experts

Helping advance science, one protein at a time.

Host–Pathogen Interactions and the Actin Cytoskeleton
Related Publications
Research Tools

August 2022

Type 3 Secretion System

Host–Pathogen Interactions and the Actin Cytoskeleton

In interacting with their human hosts, many species of pathogenic bacteria exploit the actin cytoskeleton or its associated molecular machinery, and they do so in different ways. Some manipulate it to form structures that allow them to survive on the cell surface. Others induce actin remodeling of the membrane to enter cells, and once internalized, certain species use actin polymerization to drive movement within or between host cells. Alternatively, some bacteria use actin polymers as a structural support for intracellular vacuoles, enabling them to replicate freely inside the host while providing protection from immune defenses.

Although pathogenic bacteria exhibit diverse behaviors such as these, many of them share the common characteristic of invading host tissues. They achieve this by entering cells using processes that resemble phagocytosis, macropinocytosis, or endocytosis. Various strategies are employed at the molecular level by different species, but these uptake pathways all converge on actin polymerization.[1]

Shigella vs Salmonella: Utilizing Distinct Actin Hijacking Mechanisms
Well-studied examples include the foodborne pathogens *Shigella flexneri* (bacillary dysentery) and *Salmonella enterica* serovar Typhimurium (gastroenteritis).[2] Perhaps the most direct 'actin hijacking' strategy, as used by these species, is to inject effector proteins directly into the cytoplasm of the host cell, which is achieved using a type III secretion system (T3SS). These are ATPase-driven 'syringe-like' assemblies anchored in the double membrane of Gram-negative bacteria, with a protruding 'needle' for targeted delivery of secreted proteins. To gain access to the host cell cytoplasm, a 'translocon pore' is created by inserting pore-forming proteins into the plasma membrane,[3] which then docks with the needle. The injected bacterial effectors mimic eukaryotic proteins and manipulate cellular pathways to the advantage of the pathogen. Among them, some cytoskeletal remodeling factors target actin directly, whereas others function indirectly by interacting with upstream regulatory proteins.[4]

For example, *Salmonella* translocates direct actin interactors (SipA, SipC) as well as mimetics of host guanine nucleotide exchange factors to activate Cdc42 and Rac1, thereby inducing actin polymerization. Subsequently, SptP is introduced to downregulate these activities, emphasizing the carefully orchestrated nature of the invasion process.[5] Alternatively, *Shigella* induces actin polymerization mainly through recruitment and phosphorylation of cortactin.[4] The end result for both species is the same: extensive actin-based membrane ruffling of the host cell, eventually leading to envelopment and internalization of the microbe; a process commonly referred to as the 'trigger' mechanism of invasion.

Once internalized, *Salmonella* lives inside the phagosome, whereas *Shigella* escapes and acquires actin-based motility via polarized surface expression of VirG/IcsA, a bacterial protein that sequentially recruits two host factors—N-WASP and the Arp2/3 complex—to drive actin polymerization and produce movement.[6] This generates the so-called 'comet tails' characteristic of this method of propulsion, which was first discovered for *Listeria monocytogenes* as the way it moves within and between host cells, but is now known for several species. The ActA surface protein of Listeria recruits the host Arp2/3 actin polymerization complex directly,[5] illustrating the divergence often seen between species. More recent work has uncovered a diversity of mechanisms used by motile pathogenic bacteria, which in turn produce variations in 'comet tail' ultrastructure and speed of movement.[7] This is partly due to species-specific differences in the recruitment of actin-binding accessory proteins to comet tails, including factors such as profilin, filamin, and α-actinin.[8]

Alternative Pathogens Also Commandeer Actin
Another pathogen, *Chlamydia trachomatis*, similarly invades host cells by T3SS-mediated injection of TARP, an actin nucleator and bundling protein, which promotes rapid cytoskeletal remodeling to enable bacterial uptake.[9,10] However, once internalized, *Chlamydia* exploits actin polymerization to survive and proliferate rather than using it to acquire motility. The organism transitions from its elementary body form into a replication-competent reticulate body that proliferates within a large intracellular vacuole, the surface of which is rich in actin. Assembly of this structure is regulated by the chlamydial protein InaC, which activates host RhoA to control the formation of actin scaffolds around the vacuole.[11] This stabilizes the inclusion and contributes to immune evasion.[12]
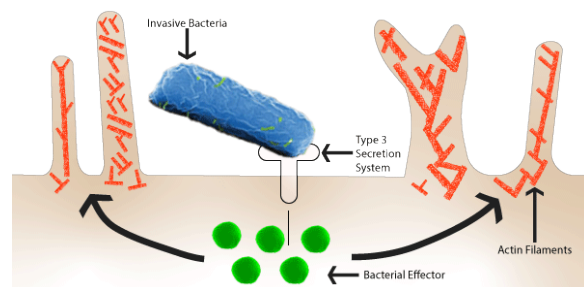

Figure Legend: Schematic of Type 3 secretion system mechanism of invasion

Continued from Page 1

In contrast, enteropathogenic and enterohemorrhagic *Escherichia coli* do not usually invade host cells, but instead reside on 'pedestal' protrusions induced by the bacteria. These structures are a hallmark of the 'attaching and effacing' lesions found in such infections, and their formation relies on actin-dependent processes, as does the accompanying effacement of gut microvilli (via actin reabsorption) that is also characteristic of the pathogenesis of these strains. All of the aforementioned processes are mediated by T3SS-delivered effectors, which are secreted in a defined sequence beginning with Tir, a transmembrane protein that is inserted into the host cell plasma membrane. Its cytosolic domains bind host factors including α-actinin[13] whereas its central extracellular domain interacts with the bacterial surface protein intimin, thereby anchoring the pathogen to the host cytoskeleton via an elaborate series of bridging interactions. Pedestal formation requires actin polymerization driven by N-WASP-mediated Arp2/3 activation, although different *E. coli* strains recruit these factors using different pathways, again illustrating the considerable diversity found among host–pathogen interactions.

Pathogen-Induced Disruption of Actin
Using an alternative mechanism, some species such as *Clostridium difficile* (diarrhea and infectious colitis) secrete a binary toxin consisting of an actin ADP-ribosyltransferase, the active component, alongside a partner protein responsible for its translocation into the cytosol by receptor-mediated endocytosis.[14,15] Once inside the cell, the enzymatic toxin component modifies G-actin at Arg177, blocking its ability to polymerize. When these ADP-ribosylated monomers are recruited to the barbed (growing) end of actin filaments, normal treadmilling is consequently inhibited. The net result is actin depolymerization, ultimately leading to cytoskeletal disarray and cell death. This is another type of immune evasion since it prevents uptake of the pathogen by professional phagocytes. Similarly, *Yersinia pseudotuberculosis* (Far East scarlet-like fever) avoids phagocytosis by injecting virulence factors that cause actin cytoskeleton collapse.[16]

Recent Discoveries and Summary
Important new discoveries continue to be made. In an intriguing recent development, it was reported that actin polymerization is necessary to open the translocon pore essential for T3SS-mediated injection of pathogenic effectors.[17] Another notable study found that *Neisseria gonorrhoeae* circumvents phagocytic killing by invading macrophages as colonies rather than as individual bacteria, which are quickly transported to lysosomes for degradation. Instead, the pathogen first establishes surface microcolonies before exploiting FMNL3, a host actin nucleating factor, to invade the macrophage and survive inside a distinct intracellular organelle surrounded by actin filaments.[18] This might explain how gonococci can evade tissue-resident immune defenses when colonizing the urogenital tract.

In a broader sense, the study of host–pathogen interactions has contributed much to our knowledge of the actin cytoskeleton and its regulation in health and disease.[5] For example, the discovery that invading bacteria can acquire actin-based motility informed related research on 'vesicle rocketing,' or intracellular vesicle trafficking, which also relies on actin-based propulsion.[8] We anticipate that ongoing advances such as these should enable the development of much-needed new therapeutics for bacterial diseases, at a time when growing resistance to existing antibiotics is already recognized as a major public health concern.

Unlabeled Actin Proteins

Unlabeled Actins	Amount	Cat . #
Actin Protein Rabbit skeletal muscle	4 x 250 µg	AKL99-A
Actin Protein Human platelet, non-muscle	2 x 250 µg	APHL99-A
Pre-formed Actin Filaments Rabbit skeletal muscle	1 x 1 mg	AKF99-A

References

1. Stradal TEB, Schelhaas M. Actin dynamics in host–pathogen interaction. FEBS Lett 2018; 592:3658–3669. https://doi.org/10.1002/1873-3468.13173.

2. Dhanda AS, Yang D, Guttman, JA. Localization of alpha-actinin-4 during infections by actin remodeling bacteria. Anat Rec 2021; 304:1400–1419. https://doi.org/10.1002/ar.24548.

3. Mota LJ, Cornelis GR. The bacterial injection kit: Type III secretion systems. Ann Med 2005; 37:234–249. https://doi.org/10.1080/07853890510037329.

4. Rottner K, Stradal TEB, Wehland J. Bacteria–host cell interactions at the plasma membrane: Stories on actin cytoskeleton subversion. Dev Cell 2005; 9:3–17. https://doi.org/10.1016/j.devcel.2005.06.002.

5. Haglund CM, Welch MD. Pathogens and polymers: Microbe–host interactions illuminate the cytoskeleton. J Cell Biol 2011; 195:7–17. https://doi.org/10.1083/jcb.201103148.

6. Suzuki T, Mimuro H, Suetsugu S, Miki H, Takenawa T, Sasakawa C. Neural Wiskott–Aldrich syndrome protein (N-WASP) is the specific ligand for Shigella VirG among the WASP family and determines the host cell type allowing actin-based spreading. Cell Microbiol 2002; 4:223–233. https://doi.org/10.1046/j.1462-5822.2002.00185.x.

7. Lamason RL, Welch MD. Actin-based motility and cell-to-cell spread of bacterial pathogens. Curr Opin Microbiol 2017; 35:48–57. https://doi.org/10.1016/j.mib.2016.11.007.

8. Hayes MJ, Shao D-M, Grieve A, Levine T, Bailly M, Moss SE. Annexin A2 at the interface between F-actin and membranes enriched in phosphatidylinositol-4,5-bisphosphate. Biochim Biophys Acta 2009; 1793:1086–1095. https://doi.org/10.1016/j.bbamcr.2008.10.007.

9. Jewett TJ, Fischer ER, Mead DJ, Hackstadt T. Chlamydial TARP is a bacterial nucleator of actin. Proc Natl Acad Sci USA 2006, 103:15599–15604. https://doi.org/10.1073/pnas.0603044103.

10. Jiwani S, Alvarado S, Ohr RJ, Romero A, Nguyen B, Jewett TJ. Chlamydia trachomatis TARP harbors distinct G and F binding domains that bundle actin filaments. J Bacteriol 2013; 195:708–716. https://doi.org/10.1128/JB.01768-12.

11. Haines A, Wesolowski J, Ryan NM, Monteiro-Brás T, Paumet F. Cross talk between ARF1 and RhoA coordinates the formation of cytoskeletal scaffolds during Chlamydia infection. mBio 2021; 12:e02397-21. https://doi.org/10.1128/mBio.02397-21.

12. Kumar Y, Valdivia RH. Actin and intermediate filaments stabilize the Chlamydia trachomatis vacuole by forming dynamic structural scaffolds. Cell Host Microbe 2008; 4:159–169. https://doi.org/10.1016/j.chom.2008.05.018.

13. Goosney DL, DeVinney R, Pfuetzner RA, Frey EA, Strynadka NC, Finlay BB. Enteropathogenic E. coli translocated intimin receptor, Tir, interacts directly with α-actinin. Curr Biol 2000; 10:735–738. https://doi.org/10.1016/S0960-9822(00)00543-1.

14. Gerding DN, Johnson S, Rupnik M, Aktories K. Clostridium difficile binary toxin CDT: Mechanism, epidemiology, and potential clinical importance. Gut Microb 2014; 5:15–27. https://doi.org/10.4161/gmic.26854.

15. Stiles BG, Pradhan K, Fleming JM, Samy RP, Barth H, Popoff MR. Clostridium and Bacillus binary enterotoxins: Bad for the bowels, and eukaryotic being. Toxins 2014; 6:2626–2656. https://doi.org/10.3390/toxins60926.

16. Alto NM, Orth K. Subversion of cell signaling by pathogens. Cold Harb Spring Perspect Biol 2012; 4:a006114. https://doi.org/10.1101/cshperspect.a006114.

17. Russo BC, Duncan-Lowey JK, Chen P, Goldberg MB. The type 3 secretion system requires actin polymerization to open translocon pores. PLoS Pathog 2021; 17(9):e1009932. https://doi.org/10.1371/journal.ppat.1009932.

18. Ivanov SS, Castore R, Rodriguez MDJ, Circu M, Dragoi A-M. Neisseria gonorrhoeae subverts formin-dependent actin polymerization to colonize human macrophages. PLoS Pathog 2021; 17:e1010184. https://doi.org/10.1371/journal.ppat.1010184

Actin Biochem Kits

Product	Assays	Cat. #
Actin Binding Protein Spin-Down Assay Biochem Kit Rabbit skeletal muscle actin	30-100	BK001
Actin Polymerization Biochem Kit (fluorescence format) Measure actin polymerization *in vitro*, contains rabbit skeletal muscle actin.	30-100	BK003
Actin Binding Protein Spin-Down Assay Biochem Kit Human platelet actin	30-100	BK013
G-Actin/F-actin In Vivo Assay Biochem Kit Measure the distribution of monomer and polymer actin	30-100	BK037

Pan Actin Antibody

Product	Amount	Cat. #
Anti-Pan Actin Mouse Monoclonal Antibody **(Clone 7A8.2.1)** **RRID# AB_2884962** **Detection of actin in human, mouse, rat, and bovine**	1 x 500 µl 1 x 125 µl	AAN02 AAN02-S