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Subversive bacteria reveal new tricks in their cytoskeleton-hijacking arsenal

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Abstract

During infection, pathogenic *Yersinia* species secrete the antiphagocytic factor YopO (or YpkA), which contains a kinase domain and a Rho GTPase guanine nucleotide dissociation inhibitor (GDI) domain. The structure of YopO in complex with actin and biochemical analyses reveal the mechanism by which YopO uses actin to activate its kinase domain and recruit, phosphorylate and deactivate actin-assembly factors implicated in phagocytic clearance of the bacterium.

Many pathogens, including bacteria^{1,2} and viruses³, use an ever-expanding arsenal of mechanisms to exploit the cytoskeleton of host eukaryotic cells for invasion, motility, replication, and avoidance of the innate immune response. Several pathogens, including *Clostridium botulinum*, *Salmonella enterica*, *Photobacterium luminescens* and *Vibrio cholerae*, secrete toxins that covalently modify actin to either block or promote polymerization⁴. Other pathogens, such as *Listeria monocytogenes*, *S. enterica* and *Shigella flexneri*, use actin assembly to promote their uptake into eukaryotic cells⁵. Finally, numerous pathogens, including vaccinia virus, *L. monocytogenes* and *Rickettsia* spp., have evolved mechanisms to harness the forces of actin polymerization for motility¹. Elegant work in this issue of *Nature Structural & Molecular Biology* by Lee *et al.*⁶ sheds light on the mechanism by which pathogenic *Yersinia* spp. use the antiphagocytic factor YopO (also known as YpkA) to recruit and deactivate actin assembly factors through phosphorylation.

Pathogenic *Yersinia* spp. (*Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*) use a syringe-like type III secretion system (T3SS) to inject six *Yersinia* outer proteins (YopE, YopT, YopH, YopO, YopM, and YopJ) into the mammalian host cell cytosol^{7,8} (Fig. 1). The Yops act in concert to neutralize the host innate immune response by inhibiting phagocytosis by macrophages and neutrophils, and downregulating the inflammatory response^{7,8}.

At least five of the Yops contain eukaryotic-like protein domains, and four (YopE, YopT, YopH and YopO) target the actin cytoskeleton, resulting in rapid actin depolymerization and enhanced virulence of the pathogen. YopE and YopT cause actin depolymerization through effects on Rho-family GTPases, which are master regulators of the actin cytoskeleton⁹, while the tyrosine phosphatase YopH dephosphorylates focal adhesion kinase (Fak), paxillin, and p130^{cas}, amongst other targets⁷.

YopO, the largest of the Yops (729-aa in *Y. enterocolitica*, the species studied by Lee *et al.*), features an N-terminal membrane-targeting domain (residues 1-89), a Ser/Thr kinase domain

(residues 108–434) and a C-terminal Rho GTPase guanine nucleotide dissociation inhibitor (GDI) domain (residues 435–729) (Fig. 1). Inside the host, YopO is targeted to the plasma membrane via the N-terminal domain¹⁰. Formation of a 1:1 complex with monomeric actin leads to the autophosphorylation of YopO at Ser-90 and Ser-95 in the loop between the membrane-targeting and kinase domains, a necessary step in the activation of the kinase^{11,12}. Since actin is only found in the host, the kinase domain remains inactive inside the pathogen. For its part, the GDI domain inhibits nucleotide exchange on RhoA and Rac1 (but not Cdc42), and disrupting these interactions through mutagenesis results in impaired YopO-induced cytoskeletal effects and attenuated virulence *in vivo*¹³.

Exogenous expression of YopO in eukaryotic cells produces a major disruption of the actin cytoskeleton^{11–15}. This appears to result from the synergistic action of all the YopO domains, since mutations disabling membrane targeting, Rho-GTPase binding or kinase activity result in reduced cytoskeleton disassembly^{11,13,14,16}. While studies using mouse infection models have sometimes produced conflicting results⁸, they generally support the notion that YopO has a substantial contribution toward the virulence of *Yersinia* spp.^{13,14,17}.

The kinase activity of YopO was discovered more than 20 years ago¹⁷, yet the mechanism by which it contributes to the neutralization of the immune response has remained elusive. The work by Lee *et al.* makes several important contributions that significantly advance our understanding of YopO function. First, the structure of YopO in complex with monomeric actin shows that the kinase and GDI domains contribute nearly equally to the interaction with actin, as it had been anticipated¹¹. Together, these two domains form a pincer-like structure that wraps around actin subdomain 4. Because this interaction interferes with inter-subunit contacts in the actin filament, the structure also explains why YopO binds monomeric, but not filamentous, actin¹¹. The interaction with subdomain 4 is rather unusual among actin-binding proteins¹⁸ and is only observed in a complex with another protein from a bacterial pathogen, toxofilin from *Toxoplasma gondii*¹⁹. The structure further explains why experiments exploring the role of the isolated kinase domain were inconclusive¹⁶; full activity of the kinase domain requires actin binding, which depends strongly on the presence of the GDI domain.

Lee *et al.* propose that the autophosphorylation loop containing Ser-90 and Ser-95 acts as an autoinhibitory peptide in the absence of actin (called here “regulatory loop”, Fig. 1). Kinase activation then occurs as a three-step process. First, the binding of actin induces a conformational change in the catalytic site, which then leads to phosphorylation of Ser-90 and Ser-95 in the regulatory loop, followed by ejection of this loop from the catalytic site for full activation of the kinase domain. This is an interesting hypothesis that remains to be tested.

The GDI domain undergoes a substantial conformational change in the complex with actin compared to its structures determined alone or in complex with Rac1¹³. When bound to actin, the C-terminal portion of the GDI domain, starting approximately at residue Ala-600 (within the so-called “backbone” helix of the GDI domain¹³), bends ~30° to close upon actin. A similar, albeit far less pronounced movement, occurs between the free and Rac1-bound structures of the GDI domain, suggesting that the backbone helix is prone to bending.

While the GTPase-binding site remains exposed in the complex with actin, and seems mostly unchanged by the interaction, one possibility that remains to be explored is whether the binding of actin allosterically modulates the affinity of GTPases for the GDI domain, concurrently with the activation of the kinase domain.

Major questions remain about the identity of the cellular substrates of the kinase domain that would explain the effects of YopO on cytoskeleton organization. A major finding by Lee *et al.* is that, at least *in vitro*, YopO uses actin not only for activation of the kinase domain, but also as bait to recruit and phosphorylate actin assembly factors that play essential roles in cytoskeleton organization, including VASP-family members, the formin mDia1, WASP and gelsolin. Coincidentally, VASP phosphorylation by YopO was recently observed in cells²⁰, supporting the notion that cytoskeletal proteins are likely to emerge as the primary targets of YopO's kinase activity. Whether such phosphorylation actually occurs as part of the infection mechanism remains to be tested. Pathogens have taught us a great deal about the function of cytoskeletal proteins, and it is possible that identification of YopO-mediated phosphorylation sites on actin assembly factors in the future will reveal novel cytoskeleton regulatory mechanisms in eukaryotes.

References

1. Welch MD, Way M. Arp2/3-mediated actin-based motility: a tail of pathogen abuse. *Cell host & microbe*. 2013; 14:242–255. [PubMed: 24034611]
2. Haglund CM, Welch MD. Pathogens and polymers: microbe-host interactions illuminate the cytoskeleton. *The Journal of cell biology*. 2011; 195:7–17. [PubMed: 21969466]
3. Taylor MP, Koyuncu OO, Enquist LW. Subversion of the actin cytoskeleton during viral infection. *Nature reviews. Microbiology*. 2011; 9:427–439. [PubMed: 21522191]
4. Aktories K, Lang AE, Schwan C, Mannherz HG. Actin as target for modification by bacterial protein toxins. *The FEBS journal*. 2011; 278:4526–4543. [PubMed: 21466657]
5. Carabeo R. Bacterial subversion of host actin dynamics at the plasma membrane. *Cellular microbiology*. 2011; 13:1460–1469. [PubMed: 21790944]
6. Lee WL, Grimes JM, Robinson RC. Yersinia effector YopO utilizes actin as bait to phosphorylate proteins that regulate polymerization. *Nature structural & molecular biology*. 2015
7. Matsumoto H, Young GM. Translocated effectors of Yersinia. *Current opinion in microbiology*. 2009; 12:94–100. [PubMed: 19185531]
8. Viboud GI, Bliska JB. Yersinia outer proteins: role in modulation of host cell signaling responses and pathogenesis. *Annual review of microbiology*. 2005; 59:69–89.
9. Hall A. Rho family GTPases. *Biochemical Society transactions*. 2012; 40:1378–1382. [PubMed: 23176484]
10. Hakansson S, Galyov EE, Rosqvist R, Wolf-Watz H. The Yersinia YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. *Molecular microbiology*. 1996; 20:593–603. [PubMed: 8736538]
11. Trasak C, et al. Yersinia protein kinase YopO is activated by a novel G-actin binding process. *The Journal of biological chemistry*. 2007; 282:2268–2277. [PubMed: 17121817]
12. Juris SJ, Rudolph AE, Huddler D, Orth K, Dixon JE. A distinctive role for the Yersinia protein kinase: actin binding, kinase activation, and cytoskeleton disruption. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:9431–9436. [PubMed: 10920208]
13. Prehna G, Ivanov MI, Bliska JB, Stebbins CE. Yersinia virulence depends on mimicry of host Rho-family nucleotide dissociation inhibitors. *Cell*. 2006; 126:869–880. [PubMed: 16959567]

14. Wiley DJ, et al. The Ser/Thr kinase activity of the Yersinia protein kinase A (YpkA) is necessary for full virulence in the mouse, mollifying phagocytes, and disrupting the eukaryotic cytoskeleton. *Microbial pathogenesis*. 2006; 40:234–243. [PubMed: 16626927]
15. Nejedlik L, Pierfelice T, Geiser JR. Actin distribution is disrupted upon expression of Yersinia YopO/YpkA in yeast. *Yeast*. 2004; 21:759–768. [PubMed: 15282799]
16. Groves E, et al. Sequestering of Rac by the Yersinia effector YopO blocks Fcγ receptor-mediated phagocytosis. *The Journal of biological chemistry*. 2010; 285:4087–4098. [PubMed: 19926792]
17. Galyov EE, Hakansson S, Forsberg A, Wolf-Watz H. A secreted protein kinase of Yersinia pseudotuberculosis is an indispensable virulence determinant. *Nature*. 1993; 361:730–732. [PubMed: 8441468]
18. Dominguez R, Holmes KC. Actin structure and function. *Annual review of biophysics*. 2011; 40:169–186.
19. Lee SH, Hayes DB, Rebowski G, Tardieux I, Dominguez R. Toxofilin from *Toxoplasma gondii* forms a ternary complex with an antiparallel actin dimer. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:16122–16127. [PubMed: 17911258]
20. Ke Y, et al. Yersinia protein kinase A phosphorylates vasodilator-stimulated phosphoprotein to modify the host cytoskeleton. *Cellular microbiology*. 2014

Figure 1. Mechanism of YopO-mediated cytoskeleton disassembly

A *Yersinia* cell establishes a first contact with a phagocyte through adhesin-receptor interactions. The bacterium deploys a syringe-like T3SS and injects several effector proteins into the eukaryotic host, including six Yops. YopO is targeted to the plasma membrane via its N-terminal domain, where it interacts with Rho-family GTPases through its GDI domain and recruits an actin monomer through interactions with both the GDI and kinase domains. Since *Yersinia* spp. inject only minute amounts of Yops into target cells where actin is the most abundant protein, actin monomer sequestration by YopO is unlikely to have a substantial effect on the eukaryotic cytoskeleton. Instead, YopO disposes of two more efficient strategies to impair the cytoskeleton: downregulation, via its GDI domain, of Rho-family GTPases that control cytoskeleton assembly, and sequestration and/or phosphorylation-dependent inactivation of cytoskeleton assembly factors. These factors include WASP-family proteins that act as Nucleation Promoting Factors (NPFs) for the Arp2/3 complex, VASP-family proteins that control filament elongation, formins that control the nucleation and elongation of non-branched actin networks, and gelsolin, a filament severing protein.