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Molecular mechanisms of *Chlamydia* escape from host cells
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Introduction: We have previously shown that *Chlamydia* spp. escape from host cells by two independent and mutually exclusive mechanisms – extrusion and lysis. Extrusion is a complex, orchestrated process that requires co-option of numerous host signaling pathways by vacuole-bound *Chlamydia*, including engagement of host actin polymerization. Lysis, in contrast, is a rapid event that consists of a series of membrane permeabilizations that originate from within the inclusion vacuole and is mediated by cysteine proteases and intracellular calcium signaling. Lysis culminates in extracellular, freely dispersed *Chlamydia*, whereas extrusion results in cell-free bacteria that remain encased by vacuole and plasma membranes. In the present study, the host signaling mechanisms that specifically contribute to chlamydial extrusion are further established. **Methods:** Single cell time-lapse confocal fluorescence videomicroscopy was performed on HeLa cells stably expressing cytosolic EGFP and infected with *C. trachomatis* LGV biovar L2. This unique approach enables detailed and dynamic visualization of the *Chlamydia* inclusion. At 72 h post-infection, infected cells were subjected to microscopy for analysis. Manipulations of host cell signaling were performed by addition of inhibitors (against actin, N-WASP, myosin II, RhoA, ROCK, MLCK) or RNA interference (RhoA, ROCK1/II, ECT2). **Results:** Extrusion was critically dependent on host actin polymerization and N-WASP activity, and included a pronounced recruitment of actin polymerization to the cytosolic face of the inclusion membrane. The contraction step of extrusion, wherein the extruded vacuole pinches off from the host cell, was shown to be mediated by actomyosin contraction and the RhoA-ROCK signaling pathway. **Discussion:** These findings support the notion that intracellular *Chlamydia* engage host cytokinesis signaling machinery to promote their cellular escape by extrusion. Although the chlamydial factors responsible for initiating this process are undefined, the overall process and underlying molecular mechanisms represent a unique mechanism of interaction between a parasitophorous vacuole and the host cell.

Keywords: Chlamydia, extrusion, exit, actin