

SPOTLIGHT

Cytosolic companionship: *Rickettsia* connects with the endoplasmic reticulum

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***Rickettsia* are bacterial pathogens known for their actin-based motility in the host cell cytoplasm. In this issue, Acevedo-Sánchez and colleagues (<https://doi.org/10.1083/jcb.202406122>) discover non-motile *Rickettsia* bacteria hijack host machinery to form stable membrane contact sites with the host endoplasmic reticulum.**

Intracellular bacterial pathogens are notorious for their stealthy and cunning lifestyles, often relying on the host cell for nutrients due to their streamlined genomes. As a result, pathogens such as tick-borne *Rickettsia* employ a range of sophisticated strategies to survive and flourish within the hostile host cell environment, including evading host immune detection, acquiring essential nutrients, manipulating immune responses, and facilitating motility and intercellular spread to ensure bacterial persistence and proliferation (1). To obtain nutrients and promote bacterial survival, *Rickettsia* often mimics or subverts host cell processes. A classic example is *Rickettsia*'s actin-based motility, where the bacteria hijacks host cell actin to form actin tails essential for cell-to-cell spread and dissemination (2).

An emerging strategy involves pathogens targeting host machinery associated with interorganelle membrane contact sites (MCSs). An MCS is a specialized region where the membranes of two organelles come in close proximity without fusing, typically within 10–30 nm (3). These sites are stabilized by protein complexes that bridge the gap between the membranes and mediate communication and exchange of materials between the organelles. MCSs play critical roles in cellular homeostasis by facilitating the transfer of lipids, ions, and metabolites, as well as coordinating signaling pathways and organelle dynamics. The vacuolar bacterial pathogens *Coxiella burnetii* and *Chlamydia trachomatis* secrete proteins that form

MCSs between the pathogen-containing vacuole and the host ER (4, 5). In both cases, the bacteria target host ER VAP proteins, which are known to mediate contact sites between the ER and mitochondria, lipid droplets, endosomes, and other host organelles. For *Coxiella* and *Chlamydia*, these MCSs are critical for regulating lipid content of the pathogen-containing vacuole, and the absence of MCSs negatively impacts bacterial replication (6, 7).

In contrast to *Coxiella* and *Chlamydia*, *Rickettsia parkeri* is a cytosolic pathogen with a characteristic actin-based motility. Because the bacteria survive free in the cytoplasm, it was a surprising discovery when Acevedo-Sánchez and colleagues observed contact sites between the bacteria and host ER (8) (Fig. 1 A). Using live cell microscopy, they observed that a small percentage of wild-type *Rickettsia* bacteria interacted with the ER in two distinct phenotypes. The majority (50–60%) were surrounded by the ER in stable “vacuole-like” structures, which often persisted over several hours. Alternatively, the bacteria were found in “protrusive” structures, where the ER associated with the bacterial actin tail. Most likely, the ER protrusive structures form when the actin-propelled bacteria interact with the ER while propelling around the crowded cytoplasm. The presence of these two phenotypes suggests that both motile and nonmotile *R. parkeri* interact with the ER, possibly through different mechanisms.

To gain further insight into the stable vacuole-like ER structures, Acevedo-Sánchez

et al. investigated a *R. parkeri* mutant lacking Sca2, a bacterial effector protein that mimics host actin nucleation factors to drive actin tail formation. In the nonmotile *sca2* mutant, most bacteria formed stable interactions with the ER, which the authors termed bacteria-ER contacts, or BERCs. Interestingly, within individual host cells, the formation of BERCs displayed an all-or-nothing phenotype: either all bacteria in the cell established stable BERCs, or none did. The underlying role for this binary behavior remains unclear, as it does not appear to affect bacterial burden and is not linked to autophagy. Further, BERCs are not cell type dependent, but thus far are *Rickettsia* specific, as they are not observed for the cytosolic pathogens *Listeria* or *Shigella*.

By light microscopy, the vacuole-like structures are a striking phenotype, with the ER wrapping around the bacteria. Transmission electron microscopy and focused ion beam scanning electron microscopy further revealed that BERCs involve the ribosome-studded rough ER. On average, close to 60% of the bacteria surface was in contact with the rough ER, at an average distance of 56 nm. This is comparable with contact sites between the rough ER and mitochondria, which coordinate energy production, protein synthesis, and the ER stress response (9).

To investigate the molecular basis for BERC formation, siRNA was used to deplete VAPA and/or VAPB, two ER proteins critical for formation of contact sites between the ER and host organelles. When *Rickettsia* infected VAPA/B-deficient cells, they were

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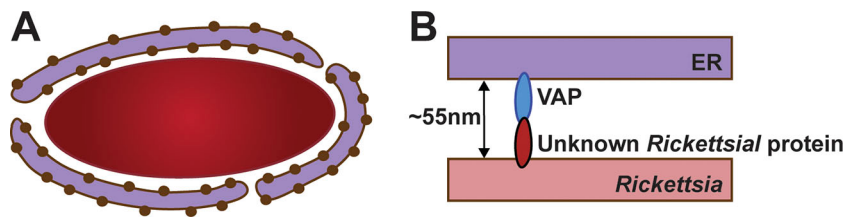


Figure 1. *Rickettsia* form MCSs with the host ER. (A) *Rickettsia*, a cytosolic bacterial pathogen, is widely recognized for its actin-based motility that is essential for cell-to-cell spread, but it can also exist in a nonmotile state. Nonmotile *R. parkeri* forms stable contact sites with the host cell rough ER, called BERCs. **(B)** BERCs involve the host cell ER VAP protein family, which bind to an unknown Rickettsial protein. The average distance between the bacteria and host ER is 55 nm.

unable to form BERCs. Further, mutating VAPA/B so they can no longer bind FFAT proteins also prevented BERC formation. This exciting finding suggests that an FFAT motif-containing protein on the bacterial surface binds VAPA/B to form *Rickettsia*-ER contacts (Fig. 1 B). In other words, *Rickettsia*-ER contact sites are bacterial driven and not a function of random interactions between the bacteria and ER.

It is not known what proteins form and maintain BERCs, though based on the requirement for VAPA/B, it is most likely a *R. parkeri* protein-containing FFAT motifs. Other key questions include when BERCs forms during infection and whether it is a host cell response or purely driven by the bacteria. Given the bimodal phenotype of the *sca2* mutant, where the bacteria in an individual cell were all either interacting with the ER or not, it is likely that BERCs are a highly regulated process influenced by both the bacteria and host cell. It is possible that BERCs serve as a host cell mechanism to restrict bacterial motility, thus decreasing bacterial dissemination.

Notably, this is the first description of a cytosolic pathogen directly interacting with host organelles through MCSs. The finding that *Listeria* and *Shigella*, other cytosolic bacterial pathogens with actin-based motility, do not form BERCs suggests this is a unique *Rickettsia* strategy. A significant question is the function of BERCs during *Rickettsia* infection, as they do not appear to be essential for *Rickettsia* growth, at least in vitro. During the replication cycle, *Rickettsia* exhibits both non-motile and motile phenotypes, with motile bacterial ranging from 2% to 17% depending on the stage of infection (10). Motility is essential for disease by facilitating cell-to-cell spread and dissemination; however, the role of non-motile bacteria and how bacteria switch between motile and nonmotile is not understood. Do BERCs participate in metabolite exchange from the ER to the bacteria, perhaps serving as an important signaling event? Are they involved in bacterial fitness in the animal, or perhaps even in the tick vector? Or do they enable the bacteria to manipulate host cell functions, such as ER stress? Addressing these questions will not only deepen our

understanding of *Rickettsia* pathogenesis but also shed light on how MCSs contribute to host-pathogen interactions more broadly. As we uncover the unique strategies employed by *Rickettsia*, we may reveal fundamental principles of intracellular survival and adaptation that extend beyond these fascinating bacteria.

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