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Cheating death: a *Coxiella* effector prevents apoptosis

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A commentary on

Inhibition of pathogen-induced apoptosis by a *Coxiella burnetii* type IV effector protein

by Luhrmann, A., Nogueira, C. V., Carey, K. L., and Roy, C. R. (2010). Proc. Natl. Acad. Sci. U.S.A. 107, 18997–19001.

Intracellular bacterial pathogens have developed strategies to subvert numerous host cell processes, often by deploying a battery of secreted proteins, termed effectors, into the cytosol. The stealthy agent of Q fever, *Coxiella burnetii*, continuously manipulates its eukaryotic host cell throughout a prolonged infectious cycle and replicates in a unique phagolysosome-like vacuole. The organism encodes a Dot/Icm type IV secretion system (T4SS) similar to that of closely related *Legionella pneumophila*, the causative agent of Legionnaires' disease (Seshadri et al., 2003). These pathogens' respective T4SSs are predicted to translocate a large number of effectors directly from the vacuole into the host cell cytosol where they interact with eukaryotic proteins to influence infection events. While over 300 *Legionella* Dot/Icm substrates have been identified (Hubber and Roy, 2010), *Coxiella* encodes few obvious homologs of these or other bacterial effectors, indicating the use of pathogen-specific repertoires. However, T4SS conservation allows the use of *Legionella* to study *Coxiella* effectors, expanding the panel of tools available for this genetically intractable organism.

Recent studies show *Coxiella* isolates encode 14 Ank proteins containing eukaryotic-like ankyrin repeat domains, 11 of which are translocated by the *Legionella* Dot/Icm T4SS (Pan et al., 2008; Voth et al., 2009). However, Ank function has remained a mystery until a recent report by Luhrmann et al. (2010) examining *Coxiella* effector-driven anti-apoptotic activity. *Coxiella* antagonizes intrinsic apoptotic death in macrophages (Voth et al., 2007) and prevents cytochrome *c* release from mitochondria to provide a stable intracellular niche for replication (Luhrmann and Roy, 2007). The pathogen also activates Akt and Erk1/2 signaling to promote survival (Voth and Heinzen, 2009). Each of these events relies on *Coxiella* protein synthesis, suggesting the organism secretes a distinct effector(s) to regulate apoptosis. Unfortunately, effector identification and characterization has been hampered by a lack of methods for *Coxiella* genetic manipulation. As an alternative, Luhrmann et al. (2010) investigated the mechanistic activity of a *Coxiella* Ank through exploration of its anti-apoptotic function in *Legionella*.

Luhrmann et al. (2010) tested the ability of four Anks (AnkA, B, F, and G) to inhibit intrinsic apoptosis. Eukaryotic cells ectopically expressing individual Anks were treated with the apoptosis-inducing agent staurosporine. Only AnkG prevented apoptosis with ~65% of AnkG-expressing cells maintaining viability. Ankyrin repeat domains mediate eukaryotic protein–protein interactions (Mosavi et al., 2004) and Dot/Icm substrates predictably bind to, and manipulate, a specific host protein(s). Therefore, Luhrmann et al. (2010) used GST pulldown and immunoprecipitation approaches to identify host-binding partners for AnkG. Interestingly, mass spectrometry analysis and confirmatory immunoprecipitation studies identified the mitochondrial inner matrix protein p32 as a specific AnkG interacting protein. p32 is a pro-apoptotic protein that binds to Hrk to promote cytochrome *c* release (Sunayama et al., 2004) and also interacts with ARF, a pro-apoptotic p53 regulatory protein (Itahana and Zhang, 2008). Thus, AnkG interaction with p32 likely precludes interaction with other pro-apoptotic mitochondrial proteins, resulting in decreased cytochrome *c* release. This is also the first known example of a bacterial protein targeting p32 to manipulate host cell survival.

Next, the authors performed a set of interesting gain of function experiments. In contrast to *Coxiella*, *Legionella* induces rapid apoptosis in some cell types, such as dendritic cells (DCs; Nogueira et al., 2009). Therefore, the authors hypothesized that *Coxiella* AnkG would provide *Legionella* with a tool to inhibit DC apoptosis and allow replication. Mouse bone marrow-derived DCs were infected with *Legionella* expressing *Coxiella* AnkG, then assessed for apoptosis. Remarkably, adding AnkG to *Legionalla*'s effector repertoire reduced DC apoptosis by ~40%. Infecting cells with *Legionella* producing truncated AnkG showed the p32-interacting region was required for inhibition of apoptosis, highlighting the functional importance of an effector binding to a host protein. Additionally, siRNA-mediated dampening of p32 expression reduced *Legionella*-triggered DC apoptosis similar to AnkG production. Collectively, the experiments performed by Luhrmann et al. (2010) provide a mechanism of AnkG anti-apoptotic activity and demonstrate the use one pathogen's effector to study another organism's intracellular activity.

Luhrmann et al. (2010) provide the first glimpse into how a *Coxiella* effector interacts with a host protein to alter a distinct infection event (**Figure 1**). This study also further underscores the differences between *Coxiella* and *Legionella*. Despite similar T4SSs, the effector repertoires of these two pathogens are highly divergent yet required for each organism's intracellular lifestyle. The intriguing results of Luhrmann et al. (2010) also foster some remaining questions about the mechanism of AnkG-mediated protection. First, does translocated AnkG traffic to mitochondria to initiate p32 interactions? AnkG could potentially bind to cytosolic p32, preventing proper localization and interaction with mitochondrial proteins. Second, is AnkG binding to p32 alone sufficient to inhibit cytochrome *c* release? Third, does AnkG influence death receptor-mediated extrinsic apoptosis? Finally, do other Anks contribute to AnkG activity? In their initial screen, the authors showed that AnkF pro-

vides modest protection from apoptosis. Perhaps AnkF works in concert with AnkG to fully protect host cells from death. This prediction is not unprecedented, as four *Legionella* anti-apoptotic effectors target different host proteins (Banga et al., 2007; Ge et al., 2009). Luhrmann et al. (2010) have defined an important role for AnkG in *Coxiella*–host cell interactions and demonstrated elegant experimental approaches that will serve as a blueprint for functional characterization of other *Coxiella* Dot/Icm substrates. It will also be interesting to see if *Legionella* can be exploited to study *Coxiella* effectors that direct other processes such as phagolysosome formation.

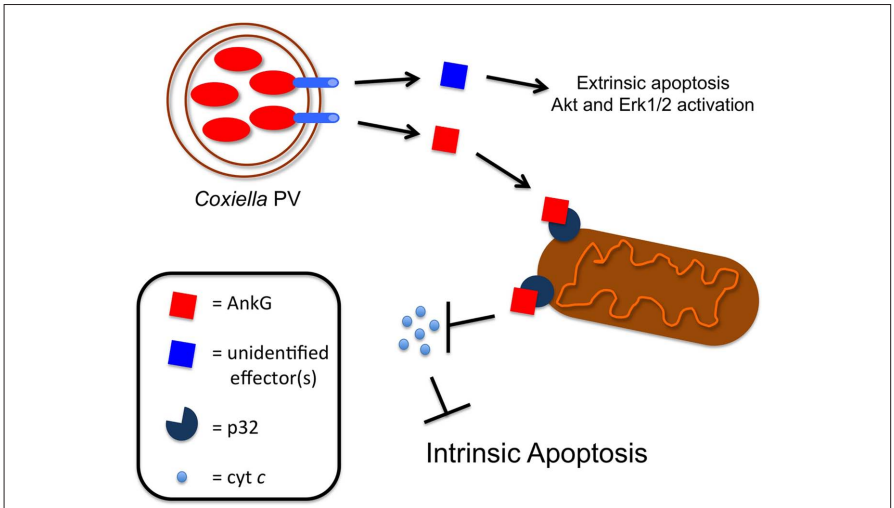

FIGURE 1 | *Coxiella* modulation of apoptosis. *Coxiella* predictably secretes numerous effectors into the host cytosol that influence events including inhibition of extrinsic and intrinsic apoptosis and activation of Akt and Erk1/2. A newly described example is AnkG, which binds specifically to host p32, presumably causing reduced cytochrome *c* (cyt *c*) release, and supplies protection from intrinsic apoptosis.

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