Analysis of Cdc42EP1 Involvement in Cdc42-required *Salmonella Typhimurium* Invasion in Intestinal Epithelium Cells

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Introduction

Cdc42 is a Rho GTPase involved in multiple regulatory pathways and is necessary in extensive actin cytoskeleton rearrangement and bacterial internalization of *Salmonella*. Cdc42 executes its regulatory function by interacting with downstream effectors, such as the largely uncharacterized and elusive family of Cdc42 effector proteins, such as Cdc42EP1. Despite extensive literature characterizing the role of Cdc42 in *Salmonella* invasions, the possible involvement of Cdc42EP1 in *Salmonella* invasions remains uninvestigated. This study aims to elucidate the role of Cdc42 effector protein, Cdc42EP1 in Cdc42-required *Salmonella* invasions in intestinal epithelium cells in order to broaden our understanding of *Salmonella* pathogenesis.

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References

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Results

1. Cdc42 and Cdc42EP1 Expression Level is Elevated in Infected Caco-2 cells

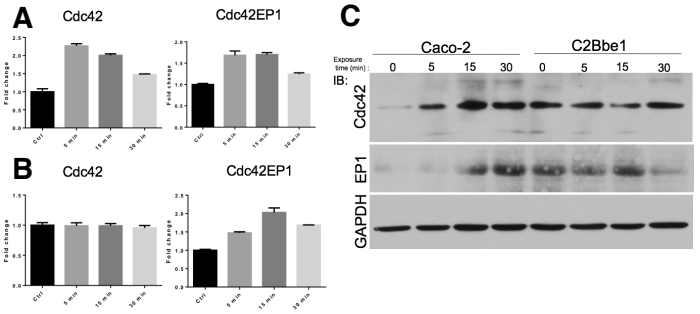

Figure 1. Cdc42 and Cdc42EP1 RNA expression levels of *Salmonella*-infected (**A**) Caco-2 and (**B**) Caco-2 brush border expressing (C2Bbe1) cells were determined by real-time PCR. (**C**) Cdc42 and Cdc42EP1 protein expression levels were examined by immunoblot analysis.

2. *S. Typhimurium* displays a greater invasive capacity in C2Bbe1 cells than in Caco-2 cells

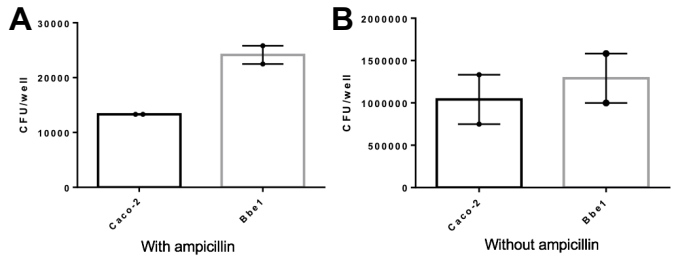

Figure 2. Cells were infected at a MOI of 250 and then incubated with gentamicin after removal of bacteria and lysed with Triton X-100. Serial dilutions of lysates were plated on XLD agar plates in (**A**) ampicillin or (**B**) ampicillin-free conditions for quantifying bacterial growth.

3. Cdc42EP1 Localizes with *Salmonella* in infected C2Bbe1 cells

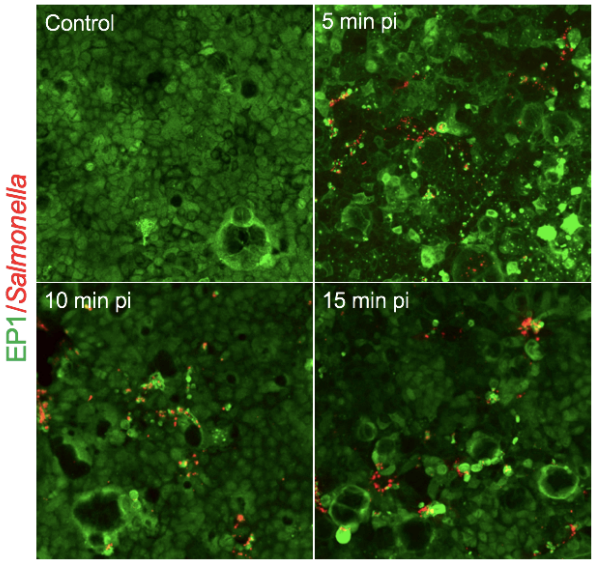

Figure 3. (**A**) C2Bbe1 cells were grown to differentiated monolayers and then infected for varying time points with *Salmonella* at a MOI of 250. Cells were fixed with 4% PFA, permeabilized with 0.2% Triton X-100, and blocked in 5% BSA-PBS. Cells were then probed for Cdc42 and *Salmonella*. Representative micrographs are shown. *S. Typhimurium* is localized with Cdc42EP1 in infected C2Bbe1 cells.

Conclusions

- *S. Typhimurium* invasion elevates Cdc42 and Cdc42EP1 expression levels in Caco-2 cells, but elevates only Cdc42EP1 expression levels in C2Bbe1 cells.
- *S. Typhimurium* has a greater invasive capacity in C2Bbe1 cells than in Caco-2 cells.
- Cdc42EP1 localizes with *S. Typhimurium* in infected C2Bbe1 cells.