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Differential localization of IcsA and IcsP in the outer membrane of Shigella flexneri

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Abstract
Differential Localization of IcsA and IcsP in the Outer Membrane of *Shigella flexneri*
Our goal is to visualize IcsP on the surface of *S. flexneri* and determine how its localization changes during growth versus stationary phases. We hypothesize IcsP will be present on the surface of *S. flexneri* differentially during growth and stationary phases of cell cycle. We hypothesize greater levels of IcsP seen during stationary phase (simultaneous with lower, more unipolar IcsA, due to IcsP mediated cleavage). During growth phase, we expect less IcsP to be present on the surface, and IcsA to be more evenly distributed around the cell surface.

Differential Localization of IcsA and IcsP in the Outer Membrane of *Shigella flexneri*

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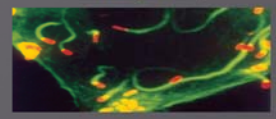

Introduction

Shigella flexneri is an intracellular pathogen that causes shigellosis, a bacterial form of dysentery. Movement within a from cell to cell is accomplished with actin filament generation at one pole of the cell which propels the bacterial cell (1). The generation of this actin "tail" is dependent on IcsA, which has been visualized at one pole of the cell (2).

IcsP is an outer membrane protease that cleaves a domain of IcsA responsible for tail formation (3). IcsA appears to be translocated uniquely to one pole where it then begins to distribute along the entire surface of the cell (see Figure 1). It has been proposed that the unipolar localization of IcsA is due to IcsA cleavage by IcsP around entire surface (4), but the actual location of IcsP has not been elucidated.

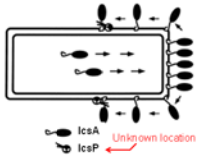

Figure 1. *Unipolar localization of IcsA during actin tail formation is proposed to be due to IcsA translocation to one pole and IcsP cleavage of diffusing IcsA around the remainder of the cell. Figure adapted from Shere, et al.*

Without IcsP, actin tail formation and mobility is enhanced occurs more quickly (5). Over-expression of IcsP limits actin tail formation and mobility (6). Therefore, IcsP is an important player in virulence and mobility regulation in *S. flexneri*, although exact roles have not been fully identified.

B galactosidase assays reveal IcsP promotors are most active during stationary phase and much less active in growth phase (Hensley, et al., unpublished data). However, so far no one has been able to visualize where IcsP is or how it interacts with IcsA in these different stages.

Thus far, the lack of information regarding localization of IcsP on the surface of *S. flexneri* is likely due to lack of an appropriate antibody and/or steric hindrance of antibody binding from LPS. To navigate these technical problems, an antibody to an exposed loop of IcsP was generated in rabbits (Pacific Immunology) and used on "rough" mutants of *S. flexneri* which have a truncated LPS (7), allowing antibodies to reach and bind native IcsP

Aim

Our goal is to visualize IcsP on the surface of *S. flexneri* and determine how its localization changes during growth versus stationary phases.

We hypothesize IcsP will be present on the surface of *S. flexneri* differentially during growth and stationary phases of cell cycle. We hypothesize greater levels of IcsP seen during stationary phase (simultaneous with lower, more unipolar IcsA, due to IcsP mediated cleavage). During growth phase, we expect less IcsP to be present on the surface, and IcsA to be more evenly distributed around the cell surface.

Materials and Methods

Bacterial Strains used:
BS109 - rough *S. flexneri*, serotype 2a
MBG340 rough *S. flexneri*, serotype 2a, lacks IcsP
BS109 pMPR402- rough *S. flexneri*, serotype 2a with inducible IcsA-GFP under L-arabinose control

Culture and Immunofluorescent Staining
Cells were grown in overnight cultures. The following morning, cells were back-diluted 1:100. Cells were grown to 2hour or 7hour time points were washed, adhered to acetone treated coverslips and fixed with 3.7% paraformaldehyde. Optical density was used to normalize the number of cells used per coverslip. Once fixed, cells were stained with rabbit anti-IcsP or rabbit anti-IcsA antibody followed by an anti-rabbit Alexa 555 secondary reagent. Cells were then visualized at 100x magnification with oil on an Olympus BX51 Immunofluorescent microscope. Images were analyzed with Adobe Photoshop.

Results and Discussion

The anti-IcsP antibody successfully stained IcsP, as 10s exposures reveal bright staining in BS109 cells, but not MBG340 cells which lack IcsP (see figure 2).

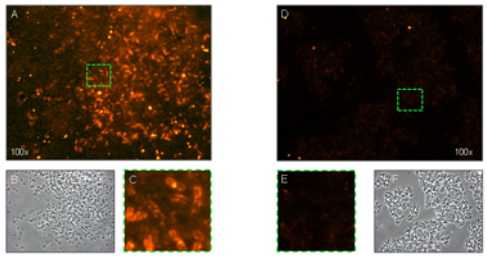

Figure 2. *Anti-IcsP antibody successfully stains BS109 cells but not MBG340 cells which lack IcsP. A-C are BS109, D-F are MBG340. A and D are 100x images with Cy3 filter; C and E are magnified areas from A and D (shown by green square). B and F are brightfield images of same viewing area of A and D.*

IcsP was seen in BS109 cells during both growth and stationary phases. Staining appears punctate, isolated into bright spots on the surface, during stationary phase but is more diffuse during growth phase (see figure 3, left panel, 1st and 3rd rows). During stationary phase, IcsA is detected on significantly fewer BS109 cells than during growth phase (see figure 3, right panel, 1st and 3rd rows) which is likely a result of IcsP cleavage during stationary phase.

These results confirm that IcsP is localized around the circumference of the bacterial cell. While it could be argued that rough mutants have greater fluidity in the outer membrane, influencing localization, our findings that IcsA still localizes in a unipolar fashion equivalent to that seen in wildtype *S. flexneri* (data not shown) gives us confidence that significant native membrane architecture is still intact.

While we were unable to visualize IcsA and IcsP on the same cell at the time of this presentation, we were able to visualize IcsP on cells which have a truncated IcsA-GFP construct. IcsA-GFP gets trapped in the cytoplasm in these cells, as GFP is too big to be moved successfully to the outer membrane. (see figure 4). Therefore, IcsA-GFP can be seen at the poles of cells, while IcsP is around the entire surface. Interestingly, in some cells IcsP seems excluded from poles where IcsA-GFP is congregated (see figure 4).

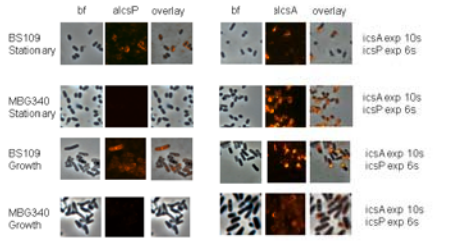

Figure 3. *Anti-IcsP (left panels) and anti-IcsA (right panels)staining of BS109 and MBG340 cells during growth phase(2hr culture) and stationary phase (7hr culture). For all images, left image is brightfield, middle image is fluorescence, and right image is overlay.*

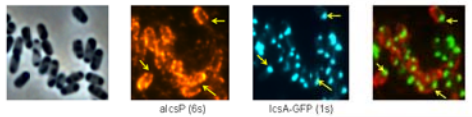

Figure 4. *Anti-IcsP staining of BS109 pMPR402 cells 1hour after induction with L-arabinose. Image on right is overlay of anti-IcsP and IcsA-GFP images. Yellow arrows show cells where IcsP seems excluded from IcsA-GFP positive pole.*

Future directions for these experiments include using commercially available IcsA antibodies with our IcsP staining technique to attempt co-staining of cells to visualize IcsA and IcsP simultaneously. MBG340 cells transformed to carry an inducible IcsP gene will also be utilized to better understand IcsP localization over time.

References

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