Rollin’ Like a Tank

Wzc’s Involvement in Polysaccharide Secretion and Gliding Motility in *Flavobacterium johnsoniae*

Grafton SMART Team: Lisa Borden, Kelsi Chesney, Elizabeth Fahey, Alex Konop, Gabrielle Kosloske, Kaleigh Kozak, Michaela Liesenberg, Chris Rose, Nick Scherzer

Teacher: Daniel Goetz
Mentor: Ryan Rhodes, Ph.D., University of Wisconsin-Milwaukee

Abstract

Cells of *Flavobacterium johnsoniae* move over surfaces using gliding motility. Gliding motility is widespread among the phylum Bacteroidetes, and several members cause disease in fish. In gliding motility, surface-exposed adhesins mediate attachment and movement of cells, and are propelled around the cell surface by motor proteins anchored in the periplasm. Evidence suggests that exopolysaccharides secreted by *F.johnsoniae* coat the substrate and provide a substrate for binding of the cell surface adhesins. Polysaccharide secretion across the outer membrane of Gram negative bacteria is facilitated by the pore-forming protein Wza and regulated by Wzc. The proteins involved in *F.johnsoniae* gliding motility are novel, and the structures have not been determined. Consequently, we modeled the tyrosine kinase domain of *E.coli* tyrosine kinase (Etk), a protein domain homologous to Wzc in *E.coli* and *F.johnsoniae*. In *E.coli*, Wzc protein forms a tetrameric oligomer, and reversible, two-step phosphorylation regulates polysaccharide secretion. In the first step, autophosphorylation of Tyr569 occurs through an intramolecular process resulting in the removal of Arg614 from the kinase active site. Unblocking the active site of Tyr569 activates the protein kinase activity of Wzc, allowing for the intermolecular phosphorylation of the tyrosine cluster on a neighboring Wzc protein. Interaction of the phosphorylated and dephosphorylated Wzc tetramer with Wza in *E.coli* results in the regulation of polysaccharide export, and researchers hypothesize that a similar mechanism controls polysaccharide secretion in *F.johnsoniae*. Elucidating the molecular mechanisms involved in gliding motility and cell adherence will advance understanding of disease pathogenesis and aid in vaccine development.

Introduction

*F. johnsoniae* is a member of the phylum Bacteroidetes and crawls over surfaces in a process known as gliding motility. Organisms that move using gliding motility move similar to the tracks on an army tank. The mechanism of gliding motility remains unknown; however, a number of genes important to the process have been identified. One such gene, wzc, encodes a protein involved in polysaccharide synthesis and export. Gliding motility is found in fish pathogens, which can be a serious problem to marine life. The more scientists know about Wzc, the greater the possibility of being able to stop the spread of pathogens in fish.

**F. johnsoniae** Infection of Russian Sturgeon

![A. Healthy](www.arkive.org)  ![B. Diseased](www.arkive.org)


Gliding Motility in *F. johnsoniae*

![Figure 2. The gliding ‘motor’, composed of Gld proteins (magenta), propels cell-surface adhesions such as SprB (black) and RemA (blue). Exopolysaccharides (orange) coat the substrate and interact with the adhesins. An F. johnsoniae bacterium pulls itself across the polysaccharides using the cell-surface adhesions. Wzc is involved in the synthesis and export of these polysaccharides.](www.arkive.org)

**Wzc plays a role in gliding motility**

![Reduced spreading on agar plates](www.arkive.org)

- **Wild-type**
- **Wzc mutated**

Figure 5. Colonies growing on an agar surface. (A) Wild-type cells “move” away from the center of the colony resulting in thin spreading edges. (B) Wzc mutant cells exhibit less colony spreading than wild type cells.

![Reduced gliding on glass slide](www.arkive.org)

- **Wild-type**
- **Wzc mutated**

Figure 6. Tracks of individual bacteria. Black lines indicate the path traveled by individual cells during 60 seconds. (A) Wild-type cells glide freely over glass. (B) wzc mutant cells move back and forth but achieve no distance.

Structure of Wzc

![Figure 3. Predicted localization of motility proteins. ‘Motor’ proteins (red) are hypothesized to move SprB and RemA (blue) adhesions around the cell. A novel secretion system (green) transports the adhesions to the cell surface. Polysaccharide synthesis and export proteins (yellow) including the inner membrane protein which interacts with Wza to export polysaccharide (orange). OM – outer membrane; CM – cytoplasmic membrane](www.arkive.org)

![Phosphorylation](www.arkive.org)

- **Inter-phosphorylation**
- **Intra-phosphorylation**

Figure 7. Intraprophosphorylation and Interphosphorylation of Wzc. Wzc is a tetramer that specifically regulates the secretion of polysaccharides and phosphorylation of the protein proceeds through a two-step mechanism. 1) Tyr569 (green star) is phosphorylated in an intramolecular reaction involving the ATP-binding Walker A motif, resulting in increased kinase activity. 2) The C-terminal Tyr cluster (pink star) is then phosphorylated in an intermolecular Tyr569-dependent reaction (*J Biol Chem.* 2002. 277(9):7127-7135). The phosphorylation of wzc is then a possible target to assist in reducing motility and possibly pathogenicity of the bacteria.

Conclusion

*F. johnsoniae* has become the standard model for studying bacteroidete gliding motility. A number of gliding organisms in this phylum are pathogenic in fish and cause substantial losses in aquaculture each year. Understanding the molecular mechanisms involved in gliding motility, including the role Wzc plays in polysaccharide secretion, will be important for developing strategies to combat these fish pathogens.

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