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Defining the Gate Domain of the Filamentous

Phage Secretin pIV

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Abstract

Secretins are a family of large outer membrane proteins with large-diameter lumens (5-10 nm). This allows them to transport bulky substrates, including folded proteins, or assembled macromolecular structures – filamentous phages and type IV pili. Many proteins exported by secretins are essential for virulence of Gram-negative pathogens. Such a large channel would ordinarily sensitize the bacterial cell to noxious agents. However, secretins do not - the presence of a mobile septum, or gate, across the lumen of the channel prevents access by noxious agents. Despite the importance of the gate in secretin function, the sequence identity of the gate residues is unknown. In this study, *in vivo* random mutagenesis was used to map amino acid residues involved in gating the filamentous phage secretin - pIV. This approach has identified 34 residues that are involved in the gating mechanism. These residues are predominantly located within the secretin homology domain and organised into two clusters; GATE1 (39 residues) and GATE2 (14 residues). A number of isolated point mutants sensitised *Escherichia coli* to bile salts and antibiotics. These findings allowed the construction of a site-directed deletion mutant of GATE2, confirming the gate function.

This thesis mapped, for the first time, a secretin gate. Given the success of the mutagenesis approach used in this thesis, the method here will be applicable to secretins of pathogenic bacteria and other outer membrane channels whose gate regions have not been determined as yet. Knowing the gate regions of “pathogenic” secretins in turn will help design secretin-targeting antimicrobials.

Foreword & Acknowledgements

“I almost wish I hadn’t gone down that rabbit-hole – and yet–and yet – it’s rather curious, you know, this sort of life” – Alice in Wonderland.

I, am an addict. I am addicted to many things, two of which are puzzles and learning. Like Alice, I find the mystery on the other side of the looking glass enticing. My ‘Rubiks-complex’ and curiosity was what drove me to research in the first place. Idealistic to say the least I wanted to ‘save the world’ and leave a mark on humanity. It is amazing what can happen during as a short time as two and a bit years to change a person. Layer by layer the harsh truths of life, society, and humanity are exposed, inducing reflection and change upon our core principles. Having observed the toxicification of idealism, I now fear for the survival of imagination. As children, we cannot wait to ‘grow-up’, at the same time, our imagination and idealism are at their peak, unbridled by the unwritten rules and nuance of the unwashed masses. Too much emphasis is placed on being normal; that we have stood silent while the evisceration of imagination takes place in our educational institutions. Instead, we are taught to sycophantically toe the party line because we have forgotten to teach ourselves how to learn; to think independently; to have an opinion and express it. These are seemingly simple things yet, without imagination, they have no hope of taking root and flourishing. What remains in their absence is an organic automaton, for without freedom of thought what are we but inconsequential meat puppets, blissfully ignorant of the ability to reach for greater purpose. To paraphrase Wayne Coyne; the only way forward for humanity is to love and let

love, if only to allow those few fearless freaks to survive and pass on the gift of imagination.

I am indebted to my supervisor, Dr. Jasna Rakonjac, for her openness and never-ending encouragement, even in the face of adversity or 'inside rain'. She gave me a home in her lab, and I can only hope she does not regret taking me on as a Masters student.

To my labmates; David Sheerin, Matt and Nick Bennett, and Dragana Gagic for their useful, humourous, and insightful discussions – thank you, I'd like to think you kept me sane, but somehow I don't think that quite fits. Without you I would have been lost.

Last, but not least, I'd like to thank my family for their support over the past few years.

Abbreviations

E. coli - *Escherichia coli*

OM - outer membrane

SPA - single particle analysis

cryo-EM - cryogenic electron microscopy

ORF - open reading frame

SOC - Super Optimal Catabolite repression media

2xYT - 2 times strength Yeast Extract, Tryptone media

IPTG - Isopropyl β -D-1-thiogalactopyranoside

X-Gal - 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

TAE - Tris-acetate-EDTA

EDTA - ethylene diamine tetraacetic acid

DMSO - Dimethyl sulfoxide

MIC - Minimal inhibitory concentration

psp operon - phage shock protein operon

Amp – Ampicillin

Cm – Chloramphenicol

BD – Becton, Dickinson and company

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