Defining the Gate Domain of the Filamentous Phage Secretin pIV

Julian Spagnuolo

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 curiousity 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.

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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- beta-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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