Extracytoplasmic Stress Responses Induced by a Model Secretin

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Abstract

Pathogenic bacteria export large proteins and protein complexes, including virulence factors, using dedicated transenvelope multiprotein machinery, collectively called secretion systems. Four of these protein export machines found in Gram-negative bacteria, type 2/3 secretion systems, filamentous phage assembly-secretion system and the type 4 pilus assembly system contain large homologous gated channels, called secretins, in the outer membrane. Secretins are radially symmetrical homomultimers (luminal diameter 6-8 nm) interrupted by an internal septum or gate. Expression of these channels imposes a fitness cost to bacteria. While stress induced by model secretin pIV has been previously investigated using microarrays, this thesis is the first RNA-seq characterisation of secretin stress responses. Furthermore, this is the first comparison of stress imposed by a closed-gate secretin (wildtype pIV), vs. an isogenic leaky-gate variant, the latter serving as a model of an open-gate substrate-secreting channel. The high sensitivity to changes in gene expression and low background noise of the RNA-seq approach have greatly expanded the known secretin stress responses to include the SoxS, CpxR and RcsB/RcsAB regulons, in addition to the known involvement of the Psp response. A synthetic lethality analysis of candidate genes in these pathways suggested that the leaky-gate secretins, besides rendering the Psp response essential for survival, also stimulate the SoxS and RcsB/RcsAB regulons for protection of the cells. Knowledge of the secretin stress expanded by this work helped identify potential targets for development of much-needed antibiotics against toxin-secreting Gram-negative bacteria.

Foreword & Acknowledgements

Bristling with anticipation, excitement and unbridled passion for science, a young man, longboard in hand, walked into an office, the first of many meetings to come. The seasoned scientist sitting across the table rebuffed his notion to save the world with ‘super-phage’ – bacteria evolve resistance too quickly. So began my journey, the start of my Masters degree which, would later weave into the, at times, perilous tale of my Doctorate.

As the months lapsed into years my ears dried, the salt hardened and increasingly I was left to my own devices, given such a free reign that, in my haste and lust, I quickly became lost in a myriad of leads, methodology and gene-regulation. I was rather lucky to have a mentor to pull me back on the path. In hindsight, I don’t think it was easy to supervise me – Jasna, Murray, my vocabulary just isn’t sufficient to express how important your support was.

Standing on the precipice of completion, I wonder just who I have done this for. Was this journey started and completed in the name of some Muse? Or was it for my own greater glory? Having stood on stage in various capacities I can safely say I do not care for glory, only acknowledgement of quality and substance. The Muses, well they disappear and reappear like the mist on the wind, I wish I could say I doubt they were important. Alas, they are attributable for waking parts of my mind that drove my journey and allow me to do the things I can. I won’t name these people, but anytime I pick up a pipette or my pen I remember each and every one. To these people – this thesis is just as much yours as mine. Live long.

Abbreviations

ABC – ATP binding cassette

ATP – Adenosine triphosphate

BH – Benjamini-Hochberg multiple testing correction

BSA – Bovine serum albumin

cAMP – Cyclic adenosine monophosphate

cDNA – Complementary DNA

Cm – Chloramphenicol

CPS – Capsular polysaccharide

CV – Coefficient of variation

DE – Differential expression

DEPC – Diethyl pyrocarbonate

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

ELISA – Enzyme linked immunosorbent assay

EM – Electron microscopy

EPS – Extracytoplasmic polysaccharide

FDR – False discovery rate

FFSS – Filamentous phage assembly/secretion system

G3P – Glucose-3-phosphate

Km - Kanamycin

IHF – Integration host factor

IM – Inner membrane

IPTG – Isopropyl β-D-1-thiogalactopyranoside

LPS – Lipopolysaccharide

NAD – Nicotinamide adenine dinucleotide

NADP – Nicotinamide adenine dinucleotide phosphate

OM – Outer membrane

OMP – Outer membrane protein

ONPG – Ortho-Nitrophenyl-β-galactoside

PCA – Principle component analysis

PEG – Polyethylene glycol

PGA – poly-β-1,6-N-acetylglucosamine

PMF – Proton motive force

POTRA – Polypeptide transport associated

PSP – Phage shock protein

$PulD_{PBS}$ – Pilotin binding domain of PulD

RCF – Relative centrifugal force

RIB – Reiterative *ihf* bacterial interspersed mosaic element

RIN – RNA integrity number

ROS – Reactive oxygen species

RNA-seq – RNA sequencing

RNA – Ribonucleic acid

rRNA – ribosomal RNA

SDS – Sodium dodecyl sulfate

SPA – Single particle analysis

sRNA – Small RNA

T2SS – Type 2 secretion system

T3SS – Type 3 secretion system

T4PS – Type 4 pilus assembly system

TCA – Tricarboxylic acid cycle

Tet – Tetracycline

UAS – Universal activation sequence

UDP – Uridine diphosphate

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