The Role of CpsC in the Regulation of *Streptococcus pneumoniae* Capsular Polysaccharide Biosynthesis

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A thesis submitted for the Degree of Doctor of Philosophy

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(June, 2012)

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Abstract

Streptococcus pneumoniae is the leading cause of pneumonia, bacteraemia (sepsis), meningitis and otitis media worldwide. It is also responsible for more deaths worldwide than any other single pathogen. A major component of pneumococcal virulence is determined by the composition of polysaccharide layer that covers the bacteria, its capsule. The composition of the pneumococcal capsule provides the basis for the classification of the pneumococcus and to date 91 structurally distinct capsular serotypes have been identified. The capsule is essential for the virulence of the pneumococcus and its primary role during pathogenesis is to act as a physical barrier preventing the interaction of cell wall bound opsonins and their cognate receptors on phagocytes, thereby preventing phagocytosis. However, during colonisation, the CPS masks adhesions suggesting the amount of CP expressed on the cell surface needs to be carefully regulated.

The regulation of capsule biosynthesis is mediated by the actions of four gene products, CpsA, B, C and D. CpsB, C and D form a series of complex interactions involved in the control of capsule subunit export and chain length regulation while CpsA has recently implicated in the ligation of capsular polysaccharide to the cell wall. A transphosphorylative event at the C-terminus of CpsD (facilitated by the presence of CpsC) results in a conformational change promoting the ligation of exported capsule subunits to the cell wall via an unknown mechanism. CpsB the de-phoshorylates CpsD resulting in a conformational change conducive to capsule export and polymerization.

CpsC has been shown to be essential for the activity of CpsD. The interactions CpsC forms with other proteins involved in capsule biosynthesis have not been fully characterized and this study sought to analyse CpsC function by performing site directed mutagenesis on *cpsC* and analysing phenotypic changes. Characterisation of these changes and further analysis was used to attempt to determine the structure and oligomeric nature of CpsC under native membrane conditions. Attempts to use computer modelling techniques to better resolve the structure and oligomeric state of CpsC were also made.

We have shown that defined single amino acid substitutions in different regions of CpsC can alter the phenotype of D39 to either mucoid or small colony phenotypes. In particular we have highlighted several domains including a region preceding and within the second transmembrane region of Cps2C that, when mutated, results in significant reductions in capsule biosynthesis. When the constructed mutants were tested in an *in vitro* adherence and invasion model (A549 cell monolayers) no consistent correlation of capsule quantity to either adherence or invasion was observed. Further characterisation of bacterial surface protein availability in these mutants did not align with either capsule quantity or the adherence and invasion results.

Structural analysis of CpsC using computer modelling and experimental approaches highlighted the sequence conservation between members of the PCP protein family but did not uncover the structural form of CpsC. However, solubilisation of CpsC in its native membrane arrangement and analysis using various proteomic methods suggests the

formation of an oligomer comprised of 4-6 monomer subunits as is consistent in the existing literature.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partnet institution responsible for the joint-award of this degree.

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[1] (Byrne et al., 2011)

Abbreviations

°C	degrees Celsius
μg	microgram/s
μl	microlitre/s
g	relative centrifugal force
A_{600}	absorbance at 600 nm
aa	amino acid/s
bp	base pairs
CFU	colony forming units
CPS	capsular polysaccharide
d	days
DNA	deoxyribonucleic acid
EDTA	ethylene-diamine-tetra-acetic-acid disodium salt
Erm^R	erthyromycin resistant
g l^{-1}	grams per litre
GRR	glycine rich region
HA	hemagglutinnin
h	hour/s
HCl	Hydrochloric acid
IPD	invasive pneumococcal disease
IPTG	isopropyl-β-D-thio-galactopyranoside
Kb	kilobase/s
kDa	kilodalton/s
kg	kilogram/s
l	litre/s
LB	Luria Bertani broth
LD_{50}	lethal dose to 50% of the population
M	molar
mg	milligram/s
min	minute/s
ml	millilitre/s
mM	millimolar
nm	nanometres
NMS	normal mouse serum
OD	optical density
O/N	overnight
ONPG	o-nitrophenyl-β-D-galactopyranoside

ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PRR	proline rich region
RT	room temperature
SDS	sodium dodecyl sulphate
s	second/s
spp	species
TAE	tris-acetate EDTA buffer
vol	volume/s
v/v	volume per volume
w/v	weight per volume

Acknowledgements

I would like to thank my supervisors, Prof. James Paton and Assoc. Prof Renato Morona, for the oppourtunity to undertake this research project and the support provided during it. It's been an interesting and elightening experience and I have you both to thank for providing encouragement, assistance and more experiments when I had no time left to do them. This project has been equally rewarding and challenging and I have appreciated your inputs at every stage.

I would like to thank all members, both past and present, of the Paton and Morona labs for their assistance, experience and friendship over the years. In particular I want to single out (in no particular order) Richard Harvey, Charlie Plumptree, Brock Herdman, Judy Morona, Jan Cook, Adam Potter, Uwe Stroeher, Campbell Strong, Magdelene (Madge) Papadopoulous, Liz Tran, Alistair Standish, Mabel Lum, Min Teh, Marcin and Kerrie Grabowicz for helping me with my work or, more often, just being good friends. It makes it very easy to work in a place with people such as yourselves to lean on when you need a hand, it makes it even easier when they are prepared to trek up to the Exeter when you need a drink.

I also want to thank Dr. Connor Thomas for providing me the template for this thesis and technical support required to use it. I didn't know how to start writing this beast and this template has been enourmously helpful.

Whilst doing this work I was given the opportunity to start writing popular science articles and for that I have to thank a few more people. Jamie Seidel, thanks for providing

the initial push to get me from thinking about writing to actually putting the words down, Clare Peddie and Melanie Bagg for helping with those first few 'Can You Believe It?' pieces that set a fire for me. Thanks must also go to Edward Michaud and Bora Zivkovic for their encouragment of my science communication and blogging and particularly to Bora for getting me a paid position at Scientific American magazine. Finally, I have to thank Thomas Tu. Without your help and our joint venture I wouldn't have started blogging and without blogging I might not have made it through the worst parts of the last few years.

A big thankyou is due for Mum and Philip for supporting me through my education, this work and this thesis. You needn't have worried about me not finishing it, I was always going to get it done, but it was very nice to know that my family was supportive of me getting to the end. Thanks must also go to my sisters, my Nan, uncles, aunties, cousins etc. for being there as a family and occasionally proding me to try and explain what I do. This all also counts to my in-laws who have accepted a weird and excitable scientist into their family and lives.

I want to thank my beautiful wife and I dedicate this thesis to her. It has been a very trying few years and you have had to put up with every high, low and in-between. For dealing with my tardiness, lateness, forgetfulness, distractedness, distance, neediness, hopelessness, excitedness and euphoria I can never thank you or express my appreciation enough. Now that it's over I promise you that you never have to endure me talking about this stuff ever again, maybe.

Lastly, I want to thank my brand new baby girl, Marnie Mills Byrne. Your birth impending and eventual birth provided the motivation to get this thesis done and out

of the way. Theres nothing quite like changing nappies whist revising chapters, and I wouldn’t change it for the world. I love you very much and hope you never feel the need to read beyond this page. Love Dad