Understanding Chromosome Dimer Resolution Systems of Pathogenic Bacteria at a Molecular Level

Stephanie Charlotte Boer B. Biotech (Hons)

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Declaration

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, 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. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

28th January, 2019

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Abstract

Although bacteria appear to be simple organisms when compared to eukaryotes, the systems by which they undergo chromosome replication and cell division are exceedingly complex. Due to the circular nature of many bacterial chromosomes, an odd number of crossing-over events that can result from homologous recombination lead to the formation of covalently linked chromosomes, known as “chromosome dimers”. These dimers need to be resolved before cell division can occur. Bacteria undergo this resolution of chromosome dimers via site-specific recombination. In *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and other bacteria, the action of FtsK, XerC and XerD produces two pairs of strand-exchange reactions which resolve chromosome dimers, thus allowing cell division to proceed. Some bacteria (e.g. *Lactococcus lactis*) and most archaea encode only a single Xer recombinase that does both strand exchanges. FtsK interacts with specific sequences that orient the protein toward “*dif*” sites, positioning the chromosome so XerC/XerD can undertake the recombination reaction. FtsK also has a role during cell division in which it pumps DNA, at an extraordinary rate, to hasten cytokinesis and chromosome segregation. The N-terminus of FtsK is primarily responsible for localisation of the protein during cell division and assembly of cell-division machinery, whilst the C-terminus forms the motor domain, responsible for translocase activity and interactions with XerD.

The work conducted herein, sought to confirm structural and functional information about FtsK from *E. coli* and *P. aeruginosa*. Functionally, it was important to identify the conserved nature of the protein by performing *in vitro* recombination experiments across both species. These recombination assays were used in downstream FtsK inhibition experiments where a suite of inhibitory compounds was designed to target its ATPase activity *in vitro* and *in vivo*. As part of structural studies, mutagenesis of the amino acids in the *E. coli* FtsKγ – XerD interaction was completed, elucidating important interactions between these two protein subunits. The final stage of experimentation was dedicated to crystallography studies, aimed at identifying a high-resolution structure of FtsK in its active state.

The results of the mutagenesis studies were published in October 2016 in a paper titled “Activation of Xer-recombination at *dif:* structural basis of the FtsKγ-XerD interaction.” This paper featured structural information of FtsKγ and XerD and how mutations in this interaction can affect its recombination effect *in vitro* and *in vivo*.