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Unlocking the M13 (f1 and fd) virion

**Investigation into the role of the pIII C-domain
of F specific filamentous bacteriophage in infection**

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Abstract

Ff filamentous bacteriophage infect male (F^+) strains of *Escherichia coli* and are assembled at the cell membranes, by a secretion-like, non-lethal process. The pIII protein, located at one end of the virion-filament, is required at both the beginning and the end of the phage life cycle. During infection, the N-terminal domains of pIII, N2 and N1, bind to the primary and secondary host receptors, F pilus and TolA protein, respectively. At the end of the life cycle, the pIII C-domain mediates the termination and release of virions. Thus, both entry and release involve structural transitions of the virus coupled to membrane transactions of the virion proteins. "Unlocking" of the highly stable virion presumably results in membrane integration during entry, whereas a reverse event, "locking" of the virion, occurs upon detachment from the membrane at termination step of assembly/secretion. Recently, it was shown that the pIII C-domain plays an active role at the step of entry. This finding implicates the C-domain of pIII in "unlocking" of the virion, presumably resulting in the exposure of the membrane anchor at the very C-terminus of pIII (Bennett & Rakonjac, 2006).

To further this work, this thesis has mapped the portion of the pIII C-domain required for infection, by constructing a set of nested deletions of the C-domain fused to the receptor binding domains N1 and N2, and then determined the infectivity of phage carrying the mutant proteins. This mapped the portion of the C-domain required for phage infection is different to that required for termination of assembly. The different requirement for entry and release suggests that the two processes are carried out by distinct mechanisms and/or depend on different sets of accessory proteins.

In addition, a system was designed for the efficient production and purification of very short virions, the length of which is 1/20 that of the wild-type f1. These short virions, called microphage, are the first step towards the structural analyses of the phage terminus cap structures, of which one contains pIII in the "locked" conformation.

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Contents

Chapter 1	Page
Literature Review	1
1.1 Introduction to filamentous bacteriophage	1
1.2 The filamentous phage genome	5
1.3 The Ff phage structure.....	11
1.3.1 Structure and role of pVIII, the major coat protein.....	11
1.3.2 The structure and role of pVII and pIX	16
1.3.3 The structure and role of pIII and pVI.....	16
1.4 The Ff phage lifecycle	22
1.4.1 Infection of host cell	22
1.4.2 Resistance to filamentous phage infection caused by pIII.....	26
1.4.3 Replication of phage within the host cell.....	26
1.4.4 The intergenic region and replication.....	31
1.4.5 The microphage-producing origin replication	32
1.4.6 Interference resistance and phage replication	35
1.4.7 The Ff phage export apparatus and phage assembly/secretion	39
1.5 Other viral mechanisms of entry	43
1.6 Aims	46
Chapter 2	
Materials and Methods	47
2.1 Bacterial strains, culture conditions and chemicals	47
2.2 Molecular biology methods	47
2.3 Construction of plasmids	48
2.4 Phage protocols	67
2.4.1 Phage stocks	67
2.4.2 Growth experiments for producing phage or phagemid particles (PPs) containing combinations of pIII mutants.....	68
2.4.3 Producer cell infection test.....	72
2.4.4 Concentration of virions by PEG precipitation.....	74
2.4.5 Purification of concentrated phage using Sarkosyl and Triton X-100	74
2.4.6 Titration of infectious phage and phagemid particles	75
2.4.7 Agarose gel electrophoresis of the phage and quantification	76
2.4.8 Protein electrophoresis and western blots.....	79
2.4.9 Growth, concentration and purification of microphage.....	79

2.5 Bootstrap Analysis	83
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Chapter 3.....Page

**Mapping of the pIII C-domain for the minimal fragment
required for phage infection..... 86**

3.1 Introduction.....	86
3.2 Experimental system for the production of	86
composite virions	86
3.3 The complete C-domain complements the assembly deficiency of the NdC83 mutation and stabilizes the NdC93-121 virions.....	88
3.4 The NdC mutants are incorporated into the virions	93
3.5 Analysis of the NdC mutant virion infectivity.....	95
3.6 A specific region of the C-domain is required for N1N2-mediated f1 infection	101
3.7 Evidence for correct folding of the N1N2 domains of pIII	102
3.8 Comparison of the infectivity of WTpIII/Cd positive control to complemented f1d3	103
3.9 Effect of N1N2 stoichiometry on opening of phage cap complex in NdC/Cd carrying phage	104
3.10 Discussion.....	105

Chapter 4.....

**Search for the pIII C-terminal domain residues involved in
infection using alanine scanning mutagenesis..... 107**

4.1 Introduction.....	107
4.2 Construction of the mutants.....	108

4.3 Phagemid particle expression system.....	108
4.4 Alanine scan mutants terminate phage assembly correctly	110
4.5 Analysis of infectivity of mutant phagemid particles	113
4.6 Summary of results	118
4.7 Secondary structure prediction	118
4.8 The ICS region is not involved in the intra-cap complex signalling.....	125
Chapter 5.....	Page
Construction of an efficient microphage-producing system	129
5.1 Introduction.....	129
5.2 Reconstruction of the microphage origin and cloning into the high copy number plasmid pCR4Blunt-TOPO.	130
5.3 A helper phage for efficient microphage production	131
5.4 Concentration and initial purification of microphage	134
5.5 Purification of microphage using native phage gel electrophoresis.....	137
5.6 Analysis of purified microphage.....	139
5.7 Visualisation of microphage by electron microscopy	143
5.8 Future uses of microphage production system for structural studies	149
5.9 Discussion of microphage production system	150
Chapter 6.....
Discussion	152
6.1 Introduction.....	152
6.2 A new model for the involvement of the C-domain of pIII in the process of phage infection.....	152

6.3 Future experiments to evaluate the role of the pIII C-domain in phage infection	156
6.4 Construction of a microphage variant of filamentous phage for structural studies	161
6.5 Effect of pVIII on phage virion length.....	161
6.6 Future modification of the microphage production system to enhance structural studies.....	167
6.7 Modification of the microphage producing system to facilitate purification	167
6.8 Comparison of f1 entry to other bacteriophage and viruses	168
Chapter 7	
Conclusion	170
Appendix	Page
Appendix 1 - General Statistic of Ff Filamentous Phage.....	173
Appendix 2 - Position of all f1 <i>gIII</i> primers used in this thesis.....	174
Appendix 3 - Protein Sequence of WTpIII and NdC mutants.....	183
Appendix 4 - I-Tasser Results	190
Appendix 5 - Reference CD	200
References	201

List of Figures	Page
Figure 1. The map of the f1 genome.....	7
Figure 2. Anatomy of Ff filamentous phage virion.	9
Figure 3. Atomic models of pVIII monomers as structured within phage virions.....	13
Figure 4. pVIII forms a shingle-like helical array around the ssDNA genome	14
Figure 5. Distal end of the Ff filamentous phage.	18
Figure 6. Three-dimensional structure of pIII N1N2 domains.....	21
Figure 7. Model of phage infection.	24
Figure 8. Overview of life cycle of Ff filamentous phage.	27
Figure 9. Secondary structure of the f1 phage intergenic sequence	29
Figure 10. Intergenic region sequence and positions of various features.....	30
Figure 11. Secondary structure of an engineered microphage origin of replication	34
Figure 12. Phage secretion, termination and release from the host cell.....	37
Figure 13. The production of cell-associated phage filaments.....	41
Figure 14. Diagram of the mutant pIII proteins used in NdC mutant experiments and positions of primers	60
Figure 15. Relative positions of the Alanine scan primers.	65
Figure 16. Method for producing mutant phagemid particles.....	70
Figure 17. Flow diagram of the production of the virions carrying combination of pIII constructs.....	73
Figure 18. A diagram describing the concept of genome equivalent.	78
Figure 19. Optimised microphage purification protocol.....	81
Figure 20. Flow diagram of the bootstrap analysis used to predict the standard deviation of infectivity.	84
Figure 21. Native phage gel electrophoresis.	91
Figure 22. Western blots of virions	94
Figure 23. Infectivity of phagemid particles containing the NdC mutants.	98
Figure 24. Infectivity of phage particles containing the NdC mutants.....	100
Figure 25. Native phage agarose electrophoresis of virions containing pIII point mutants.	111
Figure 26. Infectivity of virions containing pIII point mutants.....	117
Figure 27. Bioinformatics analysis of the C domain of pIII.	121

Figure 28. Structural model of pIII C-domain	123
Figure 29. Scenarios of pIII inter-subunit cooperation during entry.	126
Figure 30. Position of mutated codon in <i>gVIII^{am}</i> mutant used in this thesis.	133
Figure 31. Southern blot detection of microphage ssDNA from "high PEG" fractions.	135
Figure 32. Detection of the microphage by native virion agarose gel electrophoresis.	138
Figure 33. Purity and concentration of microphage	141
Figure 34. Electron micrographs of gel-purified microphage samples	145
Figure 35. High-resolution TEM and 3D atomic projection of phage filament.....	147
Figure 36. Proposed model one for f1 entry.....	155
Figure 37. Proposed genetic screen for identification of intergenic suppressor mutations in TolQRA.....	159
Figure 38. Template-dependent spatial relations between pVIII and pIII concentration gradients in the host cells.	164

List of Tables	Page
Table 1: Filamentous Phage Lifestyles	4
Table 2. PCR primers.....	49
Table 3. Plasmid	54
Table 4. Phage strains	58
Table 5. Bacterial strains list	59
Table 6. Infectivity of the NdC deletion mutant phagemid particles	97
Table 7. Infectivity phage particles containing the NdC mutants	99
Table 8. Infectivity and Relative Infectivity of Alanine Point Mutations	115
Table 9: Infective titre of phagemid particles containing WTpIII and nested deletions of the C-domain	128

List of Abbreviations

aa	-Amino acid
Amp	-Ampicillin
AP	-Alkaline phosphatase
BCIP	-5-bromo-4-chloro-3-indoxyl phosphate
bp	-Base pair
Cd	-pIII C-domain
Cm	-Chloramphenicol
Cm ^R	-Chloramphenicol Resistant
Cryo-EM	-Cryogenic electron microscopy
DNA	-Deoxyribose nucleic acid
DNase	-Deoxyribonuclease
dsDNA	-Double stranded deoxyribose nucleic acid
<i>E.coli</i>	- <i>Escherichia coli</i>
F	-F conjugative plasmid
F ⁺	- <i>E.coli</i> carrying F plasmid, also termed "male"
F ⁻	- <i>E.coli</i> not carrying F plasmid, also termed "female"
Ff	-F ⁺ specific filamentous bacteriophage of <i>E.coli</i> , including f1,fd and M13
HA	-Hemagglutinin
HBV	-Hepatitis B virus
HIV	-Human immunodeficiency virus
ICS	-Infection-competence sequence
IF	-Infective form
IPTG	-Intergenic region
IR	-Interference resistant
Kan	-Kanamycin
kb	-Kilo base
Km ^R	-Kanamycin Resistant
Km ^S	-Kanamycin Sensitive
m.o.i	-Multiplicity of infection
Nd	-pIII N-terminal domains N1 and N2

NMR	-Nuclear magnetic resonance
nt	-Nucleotide
OD	-Optical density
<i>ori</i>	-Origin of replication
PCR	-Polymerase chain reaction
PEG	-Polyethylene glycol
PONDR	-Predictors of Natural Disordered Regions
RF	-Replicative form
RNase	-Ribonuclease
Sarkosyl	-n-lauroylsarcosine, sodium salt
SDS	-Sodium dodecyl sulfate
ssDNA	-Single stranded deoxyribose nucleic acid
TBS	-Tris buffered saline
TEM	-Transmission Electron Microscopy
TFF	-Tangential Flow Filtration
WT	-Wild-type