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 termini cap structures, of which one contains pIII in the "locked" conformation.

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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
E.coli	-*Escherichia coli*
F	-F conjugative plasmid
F^+	-*E.coli* carrying F plasmid, also termed "male"
F^-	-*E.coli* not carrying F plasmid, also termed "female"
Ff	-F+ specific filamentous bacteriophage of *E.coli*, 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
ori	-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