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Unlocking of the Filamentous Bacteriophage Virion During Infection is Mediated by the C Domain of pIII

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Protein III (pIII) of filamentous phage is required for both the beginning and the end of the phage life cycle. The infection starts by binding of the N-terminal N2 and N1 domains to the primary and secondary host receptors, F pilus and TolA protein, respectively, whereas the life cycle terminates by the C-terminal domain-mediated release of the membrane-anchored virion from the cell. It has been assumed that the role of the C-terminal domain of pIII in the infection is that of a tether for the receptor-binding domains N1N2 to the main body of the virion.

In a poorly understood process that follows receptor binding, the virion disassembles as its protein(s) become integrated into the host inner membrane, resulting in the phage genome entry into the bacterial cytoplasm. To begin revealing the mechanism of this process, we showed that tethering the functional N1N2 receptor-binding domain to the virion *via* termination-incompetent C domain abolishes infection. This infection defect cannot be complemented by *in trans* supply of the functional C domain. Therefore, the C domain of pIII acts in concert with the receptor-binding domains to mediate the post receptor binding events in the infection.

Based on these findings, we propose a model in which binding of the N1 domain to the periplasmic portion of TolA, the secondary receptor, triggers *in cis* a conformational change in the C domain, and that this change opens or unlocks the pIII end of the virion, allowing the entry phase of infection to proceed.

To our knowledge, this is the first virus that uses the same protein domain both for the insertion into and release from the host membrane.

Keywords: filamentous bacteriophage; phage infection; gene 3 protein; f1 phage; phage display

Filamentous bacteriophages (Ff) are released from their host cells without lysis, by a process similar to the secretion of virulence factors or the assembly of surface filaments.[1]

F pilus-specific filamentous phage of *Escherichia coli* (f1 or fd or M13; 98% identical) have been used extensively (and interchangeably) as a model system for protein translocation and insertion into the membranes of Gram-negative bacteria.[2–5] They have also been the principal workhorse of phage display technology.[6,7] Protein III (pIII), the subject of this work, is the virion protein most commonly used as a platform for display of peptides and proteins.

The f1 virion is composed of five proteins. The major coat protein pVIII is present in about 2700 copies. A helical array of overlapping pVIII subunits forms the tube-like structure that encloses the single-stranded (ss) DNA genome; each helical turn contains five pVIII subunits.[8,9] Four to five copies each of different pairs of minor coat proteins, pVII/pIX and pIII/pVI, are located at each end of the filament.[10–12] Of the five virion proteins, four (pVIII, pVII, pIX and pVI) are short and hydrophobic, whereas pIII is relatively large and mostly hydrophilic.

Abbreviations used: Amp, ampicillin; BPB, bromphenol blue; Cm, chloramphenicol; EtBr, ethidium bromide; IPTG, isopropyl β-D-1-thiogalactopyranoside; *ori*, origin of replication; PEG, polyethylene glycol; ssDNA, single-stranded DNA; pIII, protein III; WT, wild-type.

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pIII consists of three domains, N1, N2 and C (also referred to as D1, D2 and D3), separated by flexible glycine-rich linkers. The N-terminal domains N2 and N1 bind to the primary phage receptor, the F pilus, and its secondary receptor, the TolA protein, respectively.[13–17] The N1N2 domains are tethered to the virion *via* the C domain.

Binding to both of the f1 phage receptors has been well studied at the genetic, biochemical and structural levels.[13,15–18] Little is known, however, of the entry process itself. Similarly, there is a wealth of information about phage assembly-extrusion at the genetic and biochemical level, and there is some structural information on the exit port, but again mechanistic details of the assembly process are not clear. The two processes, infection and assembly, require different sets of accessory trans-envelope protein complexes, TolQRA and pIV/pI/pXI, respectively.[14,19–24]

On the basis of genetic and biochemical studies of the C terminus of pIII, we found that a rather small part of the pIII C terminus (83 residues) is incorporated into the virion, but that a rather longer piece of pIII (93 residues) is required for release of the phage from the infected cell.[25] Protein pVI, which co-localizes with pIII at the same end of the virion, is also required for release of the phage; both proteins are also associated with the major coat protein pVIII prior to assembly into the virion. We proposed that upon incorporation into the virion, a conformational change in the C domain of pIII disrupts hydrophobic interactions of the virion with the membrane, and in this way catalyzes release of the phage.[25] If this hypothesis is correct, then entry might be the reverse of assembly-extrusion, and a conformational change in the C domain of pIII might "unlock" the virion, i.e. expose hydrophobic membrane anchors of pIII, pVI and associated pVIII subunits, which would mediate the insertion of the virion into the membrane. The two processes, entry and release, may not necessarily be symmetrical in the terms of exact residues of the C domain involved, because of difference in accessory proteins involved in the two processes, TolQRA[14] and pIV/pI/pXI,[26] respectively. Nevertheless, the involvement of the C-terminal domain of pIII in entry would implicate its active role in the infection, rather then simply linking the receptor-binding domains to the virion. Some earlier experiments bear on this point. Krebber *et al.*[27] appended the sequence encoding the single chain anti-fluorescein antibody to the N terminus of a C-terminal domain of pIII incorporated into the phage particle. To test a phage display method that aims to restore the integrity of pIII by non-covalent antibody–antigen interaction *in vitro*, they exposed the virions to the cognate antigen fluorescein conjugated to the C-terminal end of the N1N2 domain of pIII. However, the antigen–antibody interaction restored the infection efficiency only up to 10^{-4} in comparison to the virions that carried wild-type pIII. The same authors inserted β-lactamase into the full-length pIII, between the N1 and N2 or N2 and C-terminal domain of pIII and found that the infectivity of the resulting virions decreased about 100 to 1000-fold.[27] Such trends were observed by other researchers developing similar phage display systems.[28]

Here, we show explicitly that the C-terminal domain of pIII has an active and essential role in the infection and propose a model for the post-receptor binding steps of Ff phage infection.

Separation of infection and assembly/release functions of pIII

Testing of the role of the C-terminal domain of pIII in the infection required the production of phage in which the receptor-binding N1N2 domains (N) are covalently linked to a short 83 residue C-terminal fragment (C83), which is incorporated into virions but is not competent to release phage from the infected cell. This construct was named NC83 (Figure 1(a), middle diagram). Since this C-terminal fragment cannot release the phage from the cells, a second construct that lacked the N domains but expressed a complete C domain, one that is fully competent to release phage (construct C), was required for phage production (Figure 1(a), bottom diagram). These two constructs, expressed from two plasmids, were used to complement a phage that lacks gIII entirely (f1d3;[29] a flow-chart diagram of the experiment is shown in Figure 1(b)). The resulting progeny phage, which carried NC83 and C constructs (named NC83/C; Figure 1(b)) were then tested for infectivity.

As a positive control, host cells that expressed full-length (wild-type) pIII (WT) and C constructs were used to create particles with a mixture of WT and C (named WT/C; Figure 1(b)). As a negative control, host cells carrying an empty vector (used for expressing NC83 and WT) and the same C domain construct were used to create particles with C only (named C; Figure 1(b)).

The complete C domain complements the assembly deficiency of the NC83 mutant

Ff virion length is the most significant indicator of pIII-mediated release from the membranes. If pIII is not functional, the virion filaments continue to elongate, and extremely long particles, carrying more than 20 genomes, are formed.[30,31] These are initially attached to the cell; those found in the supernatant represent filaments broken off by mechanical shearing. When subjected to native virion agarose electrophoresis, these very long filaments appear as a slow-migrating smear. To determine whether the complete C domain of pIII complemented the release deficiency of NC83, the length of the virions obtained after the f1d3 infection of NC83 only-expressing cells was compared to those from cells expressing both NC83 and C (Figure 2(a)). A pattern typical of defect in phage release was observed in the samples assembled in the presence of NC83 only, and the negative control, cells lacking pIII altogether (Figure 2(a), lanes 1 and 2). In contrast, the native