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The unique two-component tail sheath of giant Pseudomonas phage PaBG

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ABSTRACT

Myoviridae bacteriophages have a special contractile tail machine that facilitates high viral infection efficiency. The major component of this machine is a tail sheath that contracts during infection, allowing delivery of viral DNA into the host cell. Tail sheaths of *Myoviridae* phages are composed of multiple copies of individual proteins. The giant *Pseudomonas aeruginosa* phage PaBG is notable in its possession of two tail sheath proteins. These tail sheath proteins are encoded by *orf 76* and *204*, which were cloned and expressed individually and together in *Escherichia coli*. We demonstrate that only co-expression of both genes results in efficient assembly of thermostable and proteolytically resistant polysheaths composed of gp76 and gp204 with approximately 1:1 stoichiometry. Both gp76 and gp204 have been identified as structural components of the virion particle. We conclude that during PaBG morphogenesis *in vivo* two proteins, gp76 and gp204, assemble the tail sheath.

1. Introduction

The complex tail structures of *Myoviridae* bacteriophages generally consist of a baseplate with tail fibers/spikes and a long non-contractile tube surrounded by a contractile sheath. During infection, following attachment of the tail fibers to the host cell, the baseplate changes its conformation, causing the sheath to contract to approximately half of its initial length. This contraction of the sheath pushes the central tube through the cellular envelope, creating a channel for ejection of genomic DNA from the capsid into the host cell (Leiman et al., 2003).

The tail sheaths of *Myoviridae* phages are known to be composed of identical protein subunits. For instance, the tail sheath of the phage T4 is composed of 138 copies of gene product (gp) 18 (Leiman et al., 2004), while the number of gp29 subunits in the longer sheath of the phage phiKZ is likely 264 (Fokine et al., 2007). Tail sheath proteins can self-assemble both *in vivo* and *in vitro* into tubular structures of variable lengths called polysheaths that resemble the contracted state of the tail sheath (Moody, 1967; Donelli et al., 1972; Kurochkina et al., 2009). Cryo-electron microscopy (EM) structures of the extended and contracted sheath of T4 and the extended sheath and polysheaths of phiKZ have been previously reported (Kostyuchenko et al., 2005; Fokine et al., 2007; Aksyuk et al., 2011). Crystal structures of T4 gp18 and phiKZ gp29, as well as of the tail sheath proteins of the prophages DSY3957 and LIN1278, have been previously solved (Aksyuk et al., 2009, 2011). Symmetry and organization of the tail was found to be similar in phiKZ and T4, as well as in many other *Myoviridae* phages, despite no significant sequence similarity in the tail proteins from the different phage groups. Moreover, the structural conservation of the sheath protein fold and similarities in the subunit arrangement during sheath contraction among different *Myoviridae* phages has been demonstrated (Aksyuk et al., 2011).

The giant bacteriophage PaBG *Pseudomonas aeruginosa* also belongs to the *Myoviridae* family and has a ~220 nm-long contractile tail. The complete genome sequence of the phage PaBG has recently been reported (Sykilinda et al., 2014). PaBG has a double-stranded 258,139 base pair DNA genome containing 308 predicted open reading frames (orfs). *Orf 76* and *204* were predicted to encode tail sheath proteins. As is already known, phage PaBG is one of two phages and both genomes encode two tail sheath proteins. *Pseudomonas putida* Lu11 is the other phage (Adriaenssens et al., 2012). It should be noted that the tail sheath proteins of each phage are related. PaBG gp76 and gp204 share 38% amino acid sequence identity, while the tail sheath proteins of Lu11 share only 31% sequence identity. None of these predicted pairs of phage-encoded tail sheath proteins have been studied experimentally, and thus, their role in phage tail morphogenesis is unknown. However, sheath assembly by two proteins has been demonstrated for the recently discovered contractile nanomashine, the bacterial type VI secretion systems (T6SSs) (Clemens et al., 2015).

In this study, two PaBG genes encoding putative tail sheath proteins were cloned and expressed both individually and together in *Escherichia coli*. The recombinant gp76 and gp204 proteins were purified and characterized by different methods to assess the contribution of each protein to assembly of the PaBG tail sheath.

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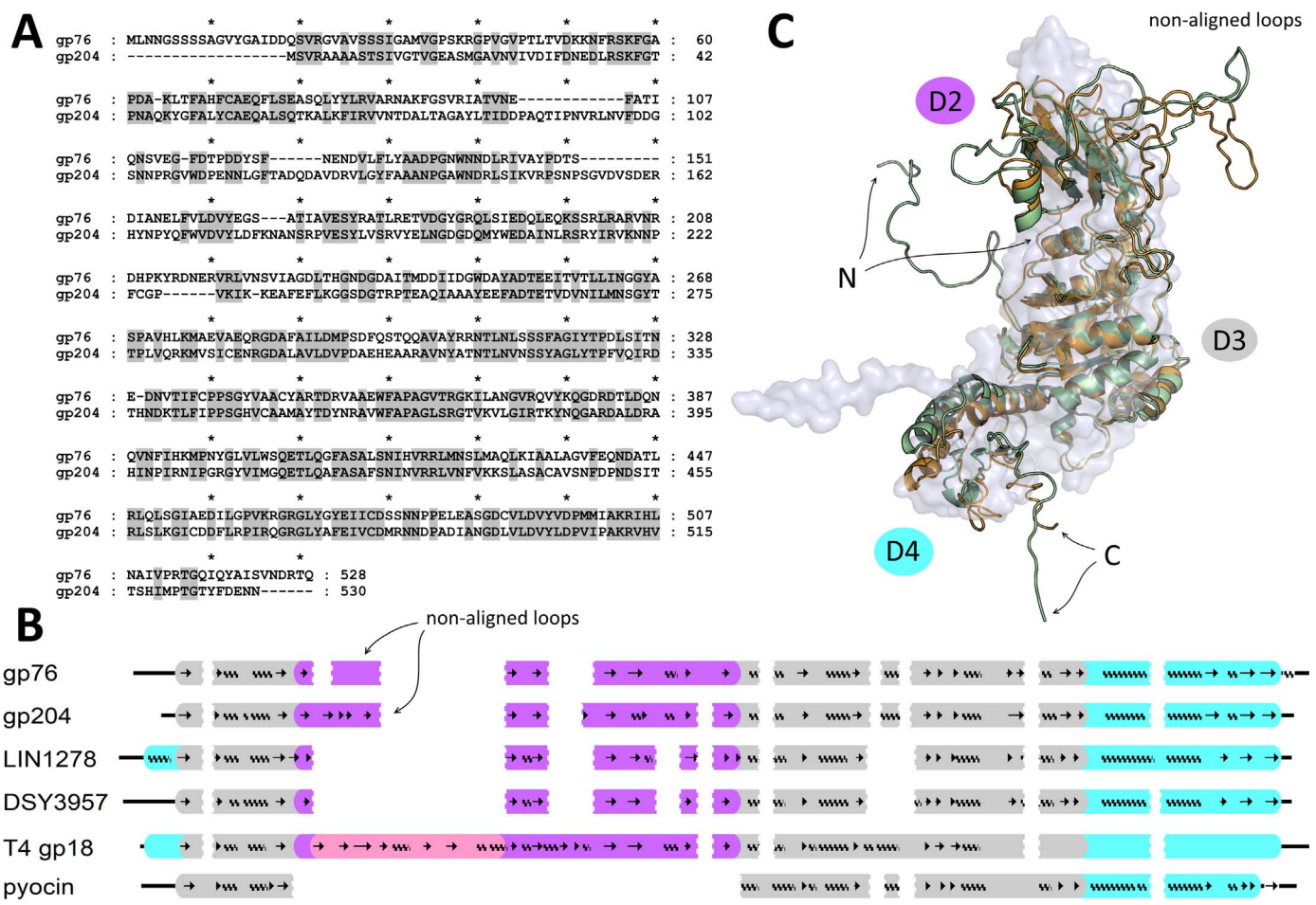

Fig. 1. Alignment of the sheath proteins. **A** – Sequence alignment of PaBG gp76 and gp204. Conserved residues are colored grey. **B** – Schematic representation of domain arrangement in the linear sequence of PaBG gp76 and gp204, the LIN1278 and DSY3957 tail sheath proteins, T4 gp18 and the pyocin sheath protein. Domains D1, D2, D3, and D4 are colored pink, violet, grey and blue, respectively; the domain numbers and colors correspond to those for T4 gp18, as was earlier defined (Aksyuk et al., 2009). Zigzags and arrows represent α-helixes and β-sheets, respectively. The secondary structure of gp76 and gp204 was predicted using HHpred. **C** – Superposition the ribbon diagrams of the predicted spatial structures of gp76 (green) and gp204 (orange) onto the transparent surface representation of the DSY3957 crystal structure (PDB ID: 3hxl). Domains D2, D3, and D4 are labeled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Results

2.1. Bioinformatics analysis of the predicted PaBG tail sheath proteins

Based on alignment of amino acid (aa) sequences, the predicted PaBG tail sheath proteins, gp76 (528 aa) and gp204 (530 aa), share 38% identity (Fig. 1A). Structural homologs were identified by searching for proteins with known tertiary structure using HHpred (Söding, 2005). Four proteins of different length, namely tail sheath proteins of the bacteriophages LIN1278 (460 aa, PDB ID: 3lml), DSY3957 (446 aa, PDB ID: 3hxl), and phi812K1-420 (587 aa, PDB ID: 5li4), as well as the sheath protein of R-type pyocin (386 aa, PDB ID: 3j9q), with a score of 280 and higher and E-value lower than 10^{-33} were found. Similarity between gp76 and gp204 of PaBG and these proteins was observed along the entire length including the termini. Among the predicted proteins, a fragment of the tail sheath protein of T4, gp18 (659 aa, PDB ID: 3foh), with E-value of 0.25 was also identified. The tail sheath proteins of the bacteriophages DSY3957, LIN1278, and T4, which are known to exhibit overall similarity in domain architecture, were selected for further analysis. Like T4 gp18, their domains are inserted into one another in a matrioshka-like manner (Aksyuk et al., 2011). Multiple sequence alignment of gp76 and gp204 of PaBG with the identified phage tail sheath proteins as well as the pyocin sheath protein was performed (scheme in Fig. 1B and full alignment in Fig. S1). The PaBG tail sheath proteins demonstrate a domain organization typical of other sheath proteins. Both gp76 and gp204 consist of three domains corresponding to domains 2, 3, and 4 of T4 gp18 and reveal the greatest extent of structural similarity with the tail sheath proteins of LIN1278 and DSY3957 because of the absence of domain 1. Instead of domain 1, there is a non-aligned loop in each of the PaBG tail sheath proteins. The absence of domain 1 and additional insertion in domain 2 is a consequence of a shorter length of their sheath proteins compared with T4 gp18. The tail sheath proteins of PaBG also revealed a structural similarity in domains 3 and 4 with the short-length pyocin sheath protein that does not have domains 1 and 2.

Based on the known crystal structures of sheath proteins, the spatial structures of gp76 and gp204 were predicted using homology modeling. With the exception of non-aligned loops, the structure of which cannot be reliably predicted, the spatial structure of the PaBG tail sheath proteins is similar. Superposition of the modeled spatial structures of gp76 and gp204 onto a crystal structure of the DSY3957 tail sheath protein is shown in Fig. 1C. Fitting results indicate that domains of both PaBG tail sheath proteins have the same folds as the corresponding regions of the DSY3957 sheath protein. Moreover, each of the PaBG tail sheath proteins fits well into the entire subunit of DSY3957. In turn, a structure of the DSY3957 tail sheath protein resembles structures of the LIN1278 sheath protein and T4 gp18 (Aksyuk et al., 2011). Since gp18 has been shown to be a repeating subunit of the phage T4 tail sheath (Aksyuk et al., 2009), each of the PaBG sheath proteins may represent an entire repeating subunit of the PaBG tail sheath.