Conformational Changes Leading to T7 DNA Delivery upon Interaction with the Bacterial Receptor*

Verónica A. González-García, Mar Pulido-Cid, Carmela García-Doval, Rebeca Bocanegra, Mark J. van Raaij, Jaime Martín-Benito, Ana Cuervo, and José L. Carrascosa

From the Structure of Macromolecules Department, Centro Nacional de Biotecnología (CSIC), Darwin 3, Cantoblanco, 28049 Madrid and Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), Cantoblanco, 28049 Madrid, Spain

Background: T7 bacteriophage infects E. coli bacteria; during this process, the tail recognizes the bacterial receptor. Results: Interactions of T7 with rough LPS trigger DNA delivery by promoting changes in the tail tube. Conclusion: E. coli rough LPS act as the receptor in vitro for T7 bacteriophage. Significance: Biotechnological application of bacteriophages as antibacterial agents demands detailed molecular knowledge of bacteriophage infection.

The majority of bacteriophages protect their genetic material by packaging the nucleic acid in concentric layers to an almost crystalline concentration inside protein shells (capsid). This highly condensed genome also has to be efficiently injected into the host bacterium in a process named ejection. Most phages use a specialized complex (often a tail) to deliver the genome without disrupting cell integrity. Bacteriophage T7 belongs to the Podoviridae family and has a short, non-contractile tail formed by a tubular structure surrounded by fibers. Here we characterize the kinetics and structure of bacteriophage T7 DNA delivery process. We show that T7 recognizes lipopolysaccharides (LPS) from Escherichia coli rough strains through the fibers. Rough LPS acts as the main phage receptor and drives DNA ejection in vitro. The structural characterization of the phage tail after ejection using cryo-electron microscopy (cryo-EM) and single particle reconstruction methods revealed the major conformational changes needed for DNA delivery at low resolution. Interaction with the receptor causes fiber tilting and opening of the internal tail channel by untwisting the nozzle domain, allowing release of DNA and probably of the internal head proteins.

Bacteriophages depend on their bacterial host for production of new viral particles. Unlike eukaryotic viruses, most bacterial viruses translocate their genetic material into the host cytoplasm, whereas the empty capsid remains on the bacterial surface (1–3). Transport of the polyanionic genome through the bacterial membranes implicates overcoming several barriers including membrane impermeability to nucleic acids and degradation by periplasmic nucleases (4). Non-tailed phages use bacterial complexes to translocate their genome (5), or they use proteins (6) or membranes (7) to form new tubular structures. The most common strategy for internalizing the bacteriophage genome is via a specialized tail complex (2, 4, 8, 9) attached through the connector protein to the capsid at the DNA packaging vertex. This complex has several functions that govern the sequence of events necessary for efficient transfer of the genetic material into the host (10, 11). The tail first closes the connector channel, preventing DNA leakage from the capsid after packaging. During infection, the tail specifically recognizes the bacterial receptor and transmits the gate-opening signal for DNA delivery. Finally, the tail conduit protects the genome during transfer to allow it to reach the bacterial cytoplasm safely.

Bacteriophage T7 belongs to the Podoviridae family; its tail is an ~2.7-MDa macromolecular complex. At least four proteins (gp8, gp11, gp12, and gp17) make up the T7 tail, which forms a central tubular structure with a closed, twisted nozzle at the bottom formed by a gp12 hexamer. This tubular assembly is surrounded by six protruding fibers specializing in receptor recognition, each of which is a gp17 trimer (12, 13). Both proteins are docked to the connector (gp8) by a dodecameric ring termed adaptor or gatekeeper (gp11). Comparative structural studies of bacteriophage tail before and after DNA ejection have been previously reported for phages φ29 (14), SPP1 (15), and T4 (16). The mechanism of DNA transport is probably best studied for T4, where the contraction of the sheath drives the tail tube through the outer membrane, forming a channel. In the case of T7, the tail is not long enough to puncture the bacterial envelope. Thus, it is postulated that some Podoviridae adopt a strategy similar to non-tailed viruses and enlarge their tail conduit by forming a new proteinaceous tubular structure when they infect Gram-negative hosts (11, 17, 18). Using cryo-electron tomography, Hu et al. visualized a temporary extension of the T7 tail during infection (18); they suggest that this extension is formed by the T7 core proteins gp14, gp15, and gp16. As these proteins are found inside the mature virus capsid, they could only form a conduit for DNA protection after transport through the tail channel and reassembly in the bacterial envelope (11, 18). The molecular mechanisms that control channel

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† These authors contributed equally to this work.

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†† To whom correspondence may be addressed: Centro Nacional de Biotecnología, CSIC, c/ Darwin 3, Cantoblanco, 28049 Madrid, Spain. Tel.: 34-915854509; Fax: 34-915854506; E-mail: acuervo@cnb.csic.es.

‡‡ To whom correspondence may be addressed: Centro Nacional de Biotecnología, CSIC, c/ Darwin 3, Cantoblanco, 28049 Madrid, Spain. Tel.: 34-915854509; Fax: 34-915854506; E-mail: jlcarras@cnb.csic.es.
opening and the precise conformational changes in the tail during this process are nonetheless unknown.

The Gram-negative bacterial envelope is composed of a peptidoglycan layer surrounded by two membranes (9, 19). During infection, the phage is thought to interact first with a surface molecule that allows correct tail orientation relative to the bacterial envelope, followed by an irreversible interaction with the same or a different receptor; this second interaction is needed to trigger opening of the tail channel (20). Viruses that infect Gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Shigella*, or *Yersinia* genus, often use lipopolysaccharide (LPS)3 as a receptor, assisted by porins or outer membrane proteins (21–23). In the case of phage T7, the identity of the bacterial receptor that triggers the conformational changes in the tail is debated. In the late 1960s, LPS from *Escherichia coli* B was reported to neutralize T7 (24). A few years later, Lindberg (21) claimed that T7 phages only infect rough strains of *E. coli* and *Shigella*, corroborating the importance of LPS in the infection mechanism. The tendency in the early 2000s was to consider LPS only a primary phage T7 receptor that mediates reversible binding to the bacterial surface, whereas another secondary receptor was suggested to be necessary for irreversible binding and infection (20, 25).

Here we used biochemical approaches to characterize the T7 particle interaction with purified LPS from *E. coli* rough strains. These studies allowed us to establish an *in vitro* ejection system for bacteriophage T7 and to carry out structural studies of the bacteriophage after ejection. Altogether these findings give new clues about the conformational changes needed in the tail to allow T7 genome transfer from the phage capsid into the bacterial cytoplasm.

**EXPERIMENTAL PROCEDURES**

**DNA Ejection Assays**—T7 (10¹¹ pfu/ml) was incubated with 250 µg/ml LPS from the rough *E. coli* EH100Ra mutant (Sigma-Aldrich) at 37 °C; aliquots were collected at 0, 15, 30, 60, 90, and 180 min, diluted in TMS (50 mM Tris-HCl pH 7.8, 10 mM MgCl₂, 100 mM NaCl), and stored on ice. Samples were then mixed with BL21 *E. coli* bacteria, plated on soft agar, and incubated (overnight, 37 °C) to determine viral titer. DNase protection assays were performed as described (26). For the reactions, the same T7/LPS ratio was incubated (37 °C) for the times indicated above.

**LPS Binding Assays**—For virus interaction assays, T7 (10¹¹ pfu/ml) was incubated with 250 µg/ml rough LPS from *E. coli* EH100 Ra mutant or smooth LPS from *E. coli* 0111:B4 (Sigma-Aldrich) (3 h, 37 °C). We incubated ~0.1 mg/ml recombinant tail complex, alone or with gp17 purified as described (13, 27), with 250 µg/ml rough LPS (2 h, 30 °C). Samples were loaded on glow-discharged copper grids covered with a thin carbon layer; negatively stained with 2% (w/v) phosphotungstic acid or uranyl acetate; and observed by EM.

**Fluorescence DNA Ejection Assays**—Fluorescence signal was recorded in a fluorimeter (Hitachi F-7000) operated at 491- and 509-nm wavelengths for excitation and emission, respectively.

**Characterization of T7 Genome Ejection**

T7 particles (~5 × 10⁹ pfu) were incubated with 0.7 µM Yo-Yo fluorophore (Invitrogen) at 37 °C for ~1000 s until the signal stabilized. Rough LPS from EH100 Ra mutant (Sigma-Aldrich) was added at 10, 20, 50, and 100 µg/ml, and the signal was recorded for at least 3000 s. As LPS forms big aggregates in solution, to calculate *Kₘ bind*, the molecular mass of the LPS was estimated to 10 kDa per chain (28). As a control, the experiment was repeated adding TMS or 10 µg/ml smooth LPS from *E. coli* 0111:B4 (Sigma-Aldrich). Curves were normalized and fitted to the double exponential equation (Equation 1) by Andres et al. (28) using the KaleidaGraph program (Synergy Software).

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DNA(t) = A₀ \times (1 - (1/k_{open} - k')\left(k_{open} \times e^{(-k' \times t)} - k' \times e^{(-k_{bind} \times t)}\right) \quad (Eq. 1)
\]

where \( k' = C_{LPS} \times k_{bind} \). The \( k_{bind} \) constant was calculated after fitting the data to a monoeponential curve at LPS saturating concentrations (50 and 100 µg/ml).

**Electron Microscopy and Image Processing**—The T7-LPS ejection reaction samples were incubated (3 h, 37 °C), applied to Quantifoil 2-µm holey carbon grids coated with a thin carbon layer, frozen in liquid ethane, and transferred to a Tecnai G20 FEG200 electron microscope (FEI) operated as described (13) at ~10,8696 magnification and 1.5–3.5-µm defocus range. Micrographs were contrast transfer function-corrected using standard XMIPP software (29); particles were manually selected with XMIPP (30) and downsamped to a factor of 4 to a final pixel size ratio of 5.5 Å/pixel. Images were classified using XMIPP reference-free clustering approach classification methods (CL2D) (31). A filtered volume of the T7 tail (32) was used as the initial model, and 1050 particles corresponding to side views were used to generate an initial three-dimensional model based on the common lines method implemented in EMAN software (33) using a restricted angle projection protocol and applying 6-fold symmetry. A final model was obtained using the XMIPP Projection Matching package (29, 34). To determine volume resolution, the particles were divided into two independent datasets (gold standard) and reprocessed. The resolution of the final reconstruction was estimated to ~20 Å. The three-dimensional model was deposited in the Electron Microscopy Data Bank (EMDB) (accession number EMD-2717). The three-dimensional reconstruction images were generated using Chimera (35).

**RESULTS**

T7 Ejects Its DNA Genome in Vitro in the Presence of Rough LPS—It has been postulated that *E. coli* rough LPS could act as the receptor for bacteriophage T7 (21, 36). To test this hypothesis, we incubated T7 phages with rough LPS. This incubation led to inactivation of the virions (Fig. 1A). After 30 min, nearly half the particles were inactivated; this percentage increased to >90% after 90 min. We used DNase protection assays to test the effect of rough LPS on T7 particles and found a decrease in the amount of DNA protected within the capsids (Fig. 1B). This finding suggested that the decrease in the percentage of infective viral particles was the result of rough LPS-induced DNA ejection. Densitometry analysis of protected DNA as a function...

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3 The abbreviations used are: LPS, lipopolysaccharide(s); cryo-EM, cryo-electron microscopy.
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To follow real-time DNA ejection at 37 °C, we performed a fluorescence experiment using the Yo-Yo fluorescent DNA binding dye. The fluorescent signal increased immediately after the addition of rough strain LPS receptor to the sample. After 1000 s, ~50% of viruses had ejected their genome and the curve stabilized in a plateau at ~5000 s (Fig. 1E). The addition of DNase led to a decrease in the fluorescence signal (data not shown). The signal was dependent on rough LPS addition as DNA was not released when LPS was not present nor when smooth strain LPS was added (Fig. 1E, gray symbols). The fluorescence intensity increase was also dependent on rough strain LPS concentration until saturation was reached at ~50 μg/ml, when 5 × 10^9 pfu of virus was used. We observed no further increase in fluorescence values when the virus was incubated with ~100 μg/ml rough LPS (Fig. 1E, black triangles). As for T5 and P22 (28, 39), T7 DNA ejection can be fitted to two first order reactions defined by two constants: 1) the first constant describes virus-receptor interaction (defined by k_{bind}), and 2) the second constant describes the channel opening reaction (defined by k_{open}). Virus-receptor interaction is LPS concentration-dependent. The data fit well to the equation proposed by Andres et al. (28). As in their case, estimation of LPS aggregates to a molecular mass of 10 kDa per chain allowed us to calculate k_{bind} (28). We obtained a k_{bind} value of ~10^4 M^{-1} s^{-1}. Fitting of the data at saturating LPS concentrations to a monoequational equation gave a k_{open} value of ~4.2 × 10^{-4} s^{-1}, in the same order of magnitude as reported for podovirus P22 (k_{open} ~ 4.5 × 10^{-4} s^{-1}) (28). The slower Podoviridae kinetics as compared with Siphoviridae (28, 39) might be due to ejection of internal head proteins as well as DNA.

The C-terminal portion of the fiber protein (gp17) is considered essential for virus-receptor interaction (12). To determine the structural component that interacts with rough LPS, we incubated rough LPS with recombinant fiber-less tail complexes and with fiber-containing tail complexes (13). EM images of the negatively stained fiber-containing samples showed the tail complex attached to the rough LPS, presumably through the fibers (Fig. 2A, left), and B, arrows), whereas no tail complexes were attached to rough LPS in the absence of fibers (Fig. 2A, right, circles). Aggregation of rough LPS in samples

of incubation time (Fig. 1B, inset) showed a reduction that is consistent with the drop of phage titer (Fig. 1A). DNA ejection in vitro in the presence of rough LPS was confirmed by EM experiments, in which negatively stained T7 particles were incubated with LPS from rough or smooth (negative control) E. coli strains (Fig. 1C). As predicted, no phases bound to the smooth LPS and no staining agent penetrated the heads, indicating that the virus remained DNA-filled (Fig. 1C, left). In contrast, incubation with LPS from rough strain E. coli showed T7 particle attachment to rough LPS layers, and the staining agent penetrated the capsid, indicating that DNA had been ejected (Fig. 1C, right).

These experiments demonstrated that incubation with rough LPS is sufficient to trigger DNA ejection in vitro and allowed us to establish an in vitro system to study T7 genome delivery. Similar findings have been reported previously for P22 (37). In our experiments with T7 bacteriophage, no proteins were observed inside the capsid after ejection (Fig. 1C, right), suggesting that the core proteins were also ejected (18). Also, we did not observe the core complex attached to the tail structure (Fig. 1C, right) as it disassembles rapidly after DNA ejection (18). Electrophoretic analysis of the ejection reaction components showed that neither the core proteins (gp14, gp15, and gp16), nor proteins gp6.7, gp7.3, and gp13 were present after DNA ejection, suggesting that they are proteolyzed after DNA translocation (Fig. 1D). Similar results have been reported in vivo (38), which implies that this degradation is essential for the infection mechanism, possibly to avoid the long-term presence of a membrane channel, which would affect cell integrity after DNA transfer to the cytoplasm. Degradation of these proteins in the in vitro reactions also suggests that one of the viral proteins might proteolyze this structure.

FIGURE 1. Rough LPS triggers T7 phage genome ejection in vitro. A, T7 particles (10^11 pfu/ml) were incubated with ~250 μg/ml rough LPS at 37 °C for the indicated times and plated. The log of the percentage of titer is plotted. Error bars indicate mean ± S.D. B, deprotection of genomic DNA from DNase digestion after incubation of T7 particles with rough LPS. The inset shows the ethidium bromide-stained 0.8% agarose gel from which the denaturing gel electrophoresis of T7 phages before the addition of rough strain LPS receptor to the sample. After 1000 s, ~50% of viruses had ejected their genome and the curve stabilized in a plateau at ~5000 s (Fig. 1E). The addition of DNase led to a decrease in the fluorescence signal (data not shown). The signal was dependent on rough LPS addition as DNA was not released when LPS was not present nor when smooth strain LPS was added (Fig. 1E, gray symbols). The fluorescence intensity increase was also dependent on rough strain LPS concentration until saturation was reached at ~50 μg/ml, when 5 × 10^9 pfu of virus was used. We observed no further increase in fluorescence values when the virus was incubated with ~100 μg/ml rough LPS (Fig. 1E, black triangles). As for T5 and P22 (28, 39), T7 DNA ejection can be fitted to two first order reactions defined by two constants: 1) the first constant describes virus-receptor interaction (defined by k_{bind}), and 2) the second constant describes the channel opening reaction (defined by k_{open}). Virus-receptor interaction is LPS concentration-dependent. The data fit well to the equation proposed by Andres et al. (28). As in their case, estimation of LPS aggregates to a molecular mass of 10 kDa per chain allowed us to calculate k_{bind} (28). We obtained a k_{bind} value of ~10^4 M^{-1} s^{-1}. Fitting of the data at saturating LPS concentrations to a monoequational equation gave a k_{open} value of ~4.2 × 10^{-4} s^{-1}, in the same order of magnitude as reported for podovirus P22 (k_{open} ~ 4.5 × 10^{-4} s^{-1}) (28). The slower Podoviridae kinetics as compared with Siphoviridae (28, 39) might be due to ejection of internal head proteins as well as DNA.

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with fiber complexes could be due to interaction of each of the six fibers with a different LPS layer (Fig. 2, A (left) and B). The results imply that T7 bacteriophage interacts with its receptor through the fibers and that this interaction triggers the conformational changes that lead to DNA delivery.

Structural Characterization of T7 DNA Delivery—In vitro ejection samples consisting of T7 and rough strain LPS were incubated (3 h, 37 °C) and then vitrified in ethane. Cryo-EM images showed a double-layered LPS filament with viruses attached through the tail complex (Fig. 3A). Incubation of T7 phages with rough LPS led to delivery of the genome and the internal core proteins; as mentioned above, we did not observe core proteins in the capsid after genome ejection reactions. Two-dimensional averages belonging to different side view classes showed the characteristic conical shape of the tail structure, with a central channel that runs from the connector structure to the LPS (Fig. 3B). The two-dimensional classes correlated well with the individual particles (Fig. 3B). We obtained the three-dimensional structure of the tail complex in the post-ejected conformation by image processing of cryo-EM data at ~20 Å (Fig. 3C). Based on the T7 tail complex structure (13), we segmented the density corresponding to the tail proteins (connector, gp8; gatekeeper, gp11; nozzle, gp12; and fibers, gp17) out of the volume, as well as the capsid protein (gp10) (Fig. 3C). We observed the characteristic shape of the connector structure, embedded in the viral capsid, as well as the N-terminal domains of the six protruding fibers attached to the conical structure and running perpendicular to the central channel (Fig. 3C). Although denaturing gel electrophoresis of the ejection reaction samples demonstrated that the gp17 protein is not degraded during the process (Fig. 1D), we were not able to see the C-terminal domain of gp17 interacting with the LPS in our reconstruction, probably due to the flexibility of the fiber structure. The nozzle complex is found at the opposite side of the connector, ending in an open channel with a 40 Å overall diameter, large enough to permit passage of the dsDNA viral genome.

Comparison of pre- and post-DNA ejection conformations (Fig. 4, A and B) permitted the definition of the conformational changes in the tail structure that correlate with DNA delivery. Some of these changes were defined using cryo-electron tomography by Hu et al. (18), who identified a change in fiber orientation. In the mature virus, the fibers are attached to the head and undergo considerable conformational change from an upward to a downward conformation, which allows interaction with the bacterial envelope (18). Although fiber flexibility did not allow us to visualize its C-terminal portion interacting with the rough LPS, single-particle reconstruction methods allowed us to increase resolution of the tail reconstruction, and thus to define more accurately the changes in the gp17 fiber N-terminal domain. After LPS interaction, the N-terminal fiber domain tilts by ~30° (Fig. 4, B and C), allowing the C-terminal domain to contact the rough LPS layer. The gp17 N-terminal domain attaches to the tubular structure through a three-lobule region (13) that could act as a hinge, allowing the long fiber structure to tilt. This change in surface contact between fiber and tubular structure might act as a sensor to trigger opening of the tail. Indeed, the DNA halted precisely at this point in
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the gatekeeper/adaptor channel (Fig. 4A, arrow), suggesting that there is a valve that retains the DNA. Long-tailed viruses have a gatekeeper valve (40), and other Podoviridae phages share the T7 DNA topology, which suggests that they use the same mechanism to secure the genome (41–43). The second major conformational change that can be observed at this resolution consists of the opening of the ejection channel by untwisting of the tail nozzle domain (compare Fig. 4, A and B).
DISCUSSION

Increased bacterial resistance to antibiotics has revived interest in using bacteriophages as antibacterial agents. Correct implementation of such treatments demands detailed molecular knowledge of bacteriophage infection. Based on these results, we describe the initial steps of T7 infection (Fig. 5). In this model, a series of conformational changes is triggered sequentially. The first interaction of the virus with the outer membrane remains elusive, but studies on bacteriophages belonging to the Podoviridae such as P-SSP7, K1E, and e15 (44) suggests that these viruses could share the same tail-opening mechanism.

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