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# Interaction of a Host Protein with Core Complexes of Bacteriophage Φ6 To Control Transcription▽

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**Bacteriophages of the family *Cystoviridae* have genomes consisting of three double-stranded RNA (dsRNA) segments, L, S, and M, packaged within a polyhedral capsid along with RNA polymerase. Transcription of genomic segment L is activated by the interaction of host protein YajQ with the capsid structure. Segment L codes for the proteins of the inner capsid, which are expressed early in infection. Green fluorescent protein (GFP) fusions with YajQ produce uniform fluorescence in uninfected cells and in cells infected with viruses not dependent on YajQ. Punctate fluorescence develops when cells are infected with YajQ-dependent viruses. It appears that the host protein binds to the infecting particles and remains with them during the entire infection period.**

Bacteriophage Φ6 is a member of the family *Cystoviridae*. The virion contains a genome of three segments (S, M, and L) of double-stranded RNA (dsRNA), which are enclosed in a polyhedral core along with polymerase molecules (20). The core is covered by a shell of a single protein species, P8, and this assemblage is enclosed in a lipid-containing membrane (5, 21). The virus propagates in plant pathogen *Pseudomonas syringae*. Genomic segment L codes for proteins P1, P2, P4, and P7, which are expressed early in infection and assemble to form the core particles (9). Transcripts of segment L along with those of segments S and M are observed early in infection. At later times, the amount of L transcript increases somewhat, while the amounts of S and M increase greatly. Most of the late-period L transcripts are found in packaged RNA, while the S and M transcripts are primarily found as free plus strands. The S and M segments code for proteins that are expressed later in infection (8). Core particles of the virus have *in vitro* transcriptase activity for genomic segments S and M (12, 13). The polymerase of Φ6 shows a preference for G as opposed to U for the second nucleotide in its transcripts (1, 19). Segments S and M begin with GG, while segment L begins with GU. Incubation with host protein YajQ results in the transcription of segment L (15). Previous work has shown that productive infection is dependent upon the presence of YajQ in the host cells and that the protein binds to P1, which is the major structural protein of the virus core particle (15). Mutants of Φ6 that are not dependent on YajQ were isolated and were shown to have changes in P1 and in P2, the virion polymerase. Our working model for the activity of YajQ is as follows. The virus attaches to the host type IV pilus, which retracts so as to bring the virion to the surface of the outer membrane, where the viral membrane fuses so as to allow the nucleocapsids to enter the periplasmic space. The virion muramidase P5 digests a pathway in the cell wall so as to allow the entry of the nucleocapsid into the cell. Protein P8 is removed from the particle by an unknown mechanism and is eventually digested (16). The core particle is capable of transcribing genomic segments S and M, but it is covered by YajQ, which activates the transcription of genomic segment L. The amount of YajQ in the cell is limited, and as new particles are formed and filled, a few are covered by YajQ, but most of the new core particles are covered by protein P8 after minus-strand synthesis on the templates of packaged transcripts. Transcription of L continues during the late period of infection, but most of the L transcript is found in packaged particles and little L transcript is seen as single-stranded RNA (15).

In this study, we attempted to visualize the *in vivo* interaction of YajQ with virus particles to facilitate an understanding of the amount and the extent of binding, the timing, and the specificity of the interaction. The interaction of YajQ with core particles was originally apparent when it was found associated with carrier state particles. The amount of the protein associated with core particles was difficult to measure, since the affinity of the interaction was diminished by salt concentrations necessary for the maintenance of the structure of filled core particles (15). To that end, we have constructed green fluorescent protein (GFP) fusions of YajQ so that the interaction of YajQ with virus particles *in vivo* could be seen.

The *gfp* gene was copied from plasmid ED430 (4) by using oligonucleotides 588 and 1255, with the sequences CCCGGA TCCTAAGAAGGAGATATACATATGAGTAAAGGAGA AGAACTTTTCAC and CCCCCTGCAGTTTGTATAGTTC ATC, respectively. The PCR product was inserted into plasmid pLM350 with a 5′ BamHI site and a 3′ PstI site to form plasmid pLM3653. Plasmid pLM350 is a shuttle vector for *Escherichia coli* and pseudomonads derived from plasmid pLM254 (10). The *yajQ* gene was copied from plasmid pLM3556 (15) by using oligonucleotides 1256 and 1258, which placed a PstI site and a His tag sequence at the 5′ end and a HindIII site at the 3′ end. The sequences were CCCCCTGC AGCACCACCATCATCACCAC for 1256 and CCCCAAGC TTAGTCGCGGAAGTTGTT for 1258. The resulting PCR

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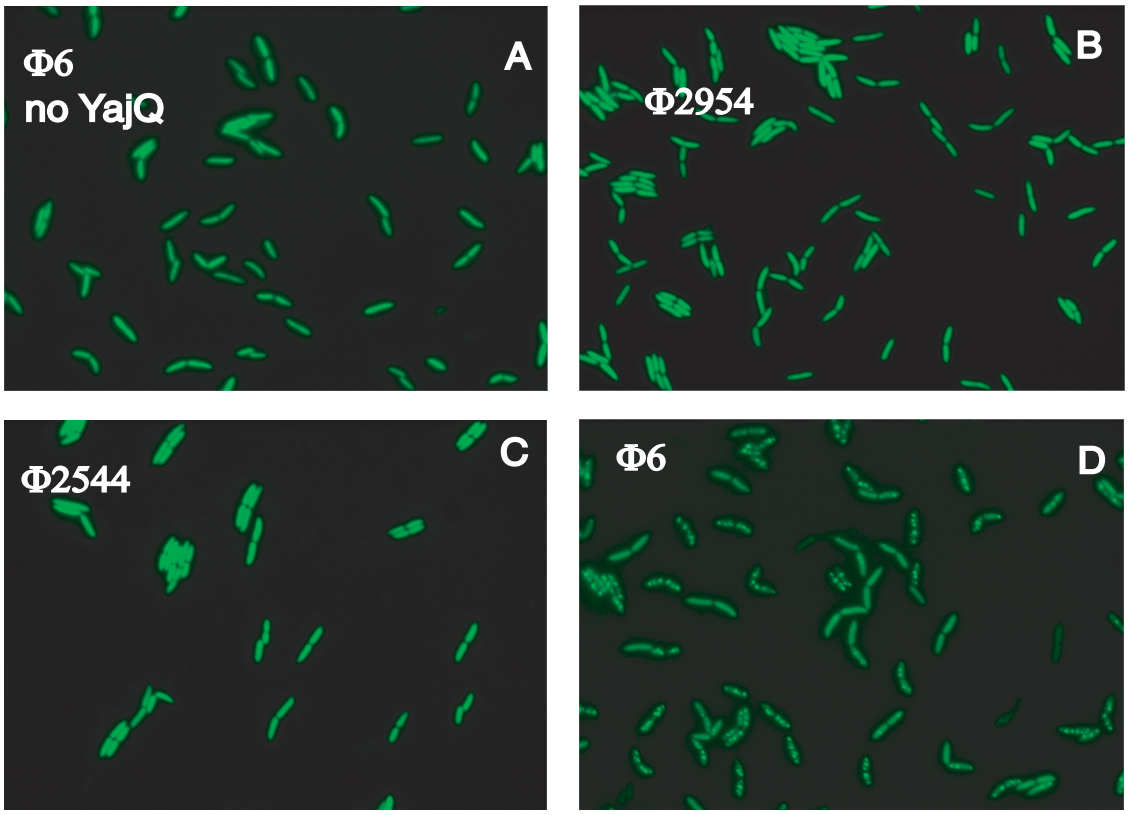


FIG. 1. Images of cells carrying plasmids expressing green fluorescent protein (GFP) or N-terminal GFP fusions with YajQ. Cells infected with Φ6 showed a punctate pattern at 75 min after adsorption (D), while cells infected with Φ2954 (B) or Φ2544 (Φ13 with M segment of Φ6) (C) showed uniform fluorescence. (A) Normal cells infected with Φ6 but lacking the fusion protein showed uniform fluorescence. Cells were deposited on 1.2% agarose pads containing LB medium. Images were collected in a Nikon 90i microscope using a TIRF Plan Neo-Fluor 100× oil immersion objective (numerical aperture, 1.45). The filter set was BrightLine GFP-3035B-NTE-ZERO from Semrock Optical. Version 4 of the Volocity application (Improvision) was used for the acquisition and quantification of fluorescence intensities.

product was inserted into pLM3653 by using a 5′ PstI site and a 3′ HindIII site to form plasmid pLM3654. Plasmid pLM3654 was transferred to strain LM4470, which is *P. syringae* rough-lipopolysaccharide (LPS) strain LM2489 with a knockout of gene *yajQ*. The resulting strain is LM4622. Plasmid pLM3653, which codes for GFP without the fusion to YajQ, was transferred to strain LM2489 to make strain LM4624.

Strain LM4624 is a *P. syringae* strain carrying a plasmid that expresses GFP that is not fused to any other protein and shows uniform fluorescence even during infection with Φ6 or other members of the *Cystoviridae* (Fig. 1A). Strain LM4622 expresses a fusion protein that contains GFP at its N terminus and YajQ at its C terminus. The chromosomal *yajQ* gene is knocked out, but the plasmid expressing the fusion protein is able to complement the knockout with respect to the replication of Φ6 and its close relatives. Infection of strain LM4622 with phages such as Φ2954 and Φ2544 (which is Φ13 with the host attachment proteins of Φ6), which are distantly related to Φ6, did not change the uniform fluorescence of the cells (Fig. 1B and C). Neither of these phages is dependent upon YajQ for plaque formation (15). However, infection with Φ6 or Φ9 (11), a close relative, results in the appearance of punctate fluorescence (Fig. 1D and 2A). The number of particles entering cells when exposed to high multiplicities of virus is limited due to the attachment to the pili. At high multiplicities of infection, only three or four virions enter the cells and about 25% of the cells are not infected (16). Cells of LM4622 showed one to four fluorescent spots, and these developed during the first 30 min of the infection (Fig. 3). It might be that the period before the punctate pattern emerges is due to the time for the P8 shell to be removed. Infection with a mutant of Φ6 that has a deletion in gene 2, coding for the polymerase, resulted in one to three punctate forms (not shown). Since this phage is not capable of producing new particles filled with dsRNA, it is clear that the punctate forms are due to the infecting phage particles and that these forms are composed of single core particles. Particles that do not contain dsRNA do not bind YajQ *in vitro* (15). The fluorescent spots remained throughout the infection and moved slightly. The yield of Φ6 in a normal infection is several hundred particles per cell, but the number of core particles that are covered by P8 or without this middle shell at any given time has not been determined. On the basis of the small number of punctate forms and their stability, we propose that YajQ remains with the infecting particles and that only a few of the new particles have YajQ on them. The amount of YajQ in each cell would limit the number of particles that can bind substantial amounts of the protein. This would explain why early transcription of L is followed by late transcription primarily of S and M. Infection by a Φ6 virion with the attachment proteins of Φ13 results in a larger initial

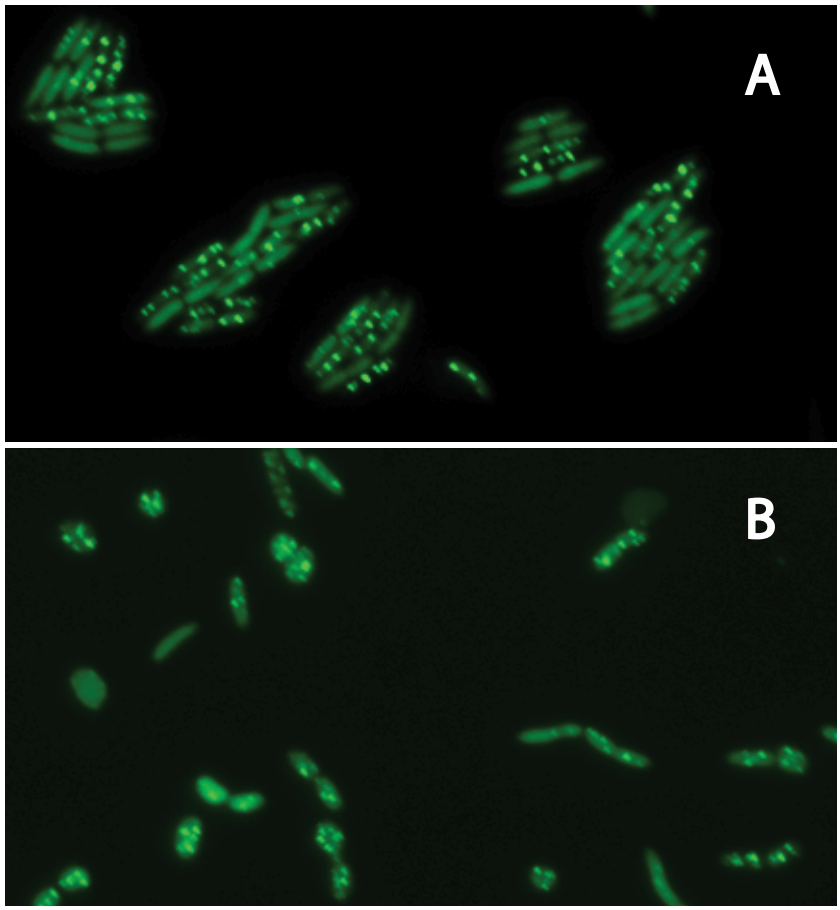


FIG. 2. (A) Infection of LM4622 with Φ9, a close relative of Φ6. (B) Infection of LM4622 with Φ2554, a derivative of Φ6 with the host attachment proteins of Φ13 enabling binding to rough LPS and consequently an increased number of entering particles and some lysis from without.

number of punctate forms (Fig. 2B). Φ13 attached to the rough LPS, and at high multiplicities of infection, the number of particles entering each cell was higher than that in the case of Φ6. It was even possible to see signs of premature lysis from without in these cases.

We determined the ratio of fluorescence in whole, uninfected cells to that of individual punctate forms. It appears that the amount of the YajQ fusion protein per cell is about six times that of the amount bound to a single particle. In addition, we determined the amount of GFP-YajQ fusions per cell by comparing the strength of the band produced by cells in a Western reaction to the strength of the band produced by dilutions of purified protein whose concentrations were determined by measuring optical density. In this case, we found that cultures of LM4622 contained an average of 1,500 molecules per cell. We suggest that approximately several hundred molecules of the YajQ fusion molecules are bound to each particle. This does not seem to be enough molecules to form a complete shell around the particle. The shell formed by protein P8 has an estimated 600 molecules (7). However, it seems clear that the number of molecules is substantial enough to cause a conformational change in the structure of the core particle.

Temporal regulation of gene expression is an important feature of bacteriophage infection programs. This is manifested by regulation of transcription or by regulation of message stability (14, 15). We have found that the replication of bacteriophage Φ6 is dependent upon the presence of host protein YajQ, a protein of unknown function that is highly conserved in Gram-negative bacteria (17). YajQ is necessary for the activation of the transcription of genomic segment L *in vitro* as well as *in vivo* (15). The mechanism of this activation is currently a mystery in that the protein binds to the major structural protein P1 of the viral core, yet it changes the activity of the polymerase which is inside the core (18). There are, however, precedents for such activity. In the case of Φ6, we have shown that polymerase is inactive during the packaging of the plus-strand transcripts of the genome until all three are inside the core (3). According to the working model, the expansion of the core changes the conformation of the polymerase so as to turn it on. In the case of rotavirus, the core particle that contains polymerase and genomic segments is not activated for transcription until viral protein VP6 binds and forms a shell structure around the core (2). In the case of reovirus, transcription is not activated until delta protein is removed from the entering core particle by the activity of host protein Hsc70 (6). Although we had found YajQ associated with viral core particles and done studies of the binding of YajQ to core particles, we could not be sure that substantial amounts of the

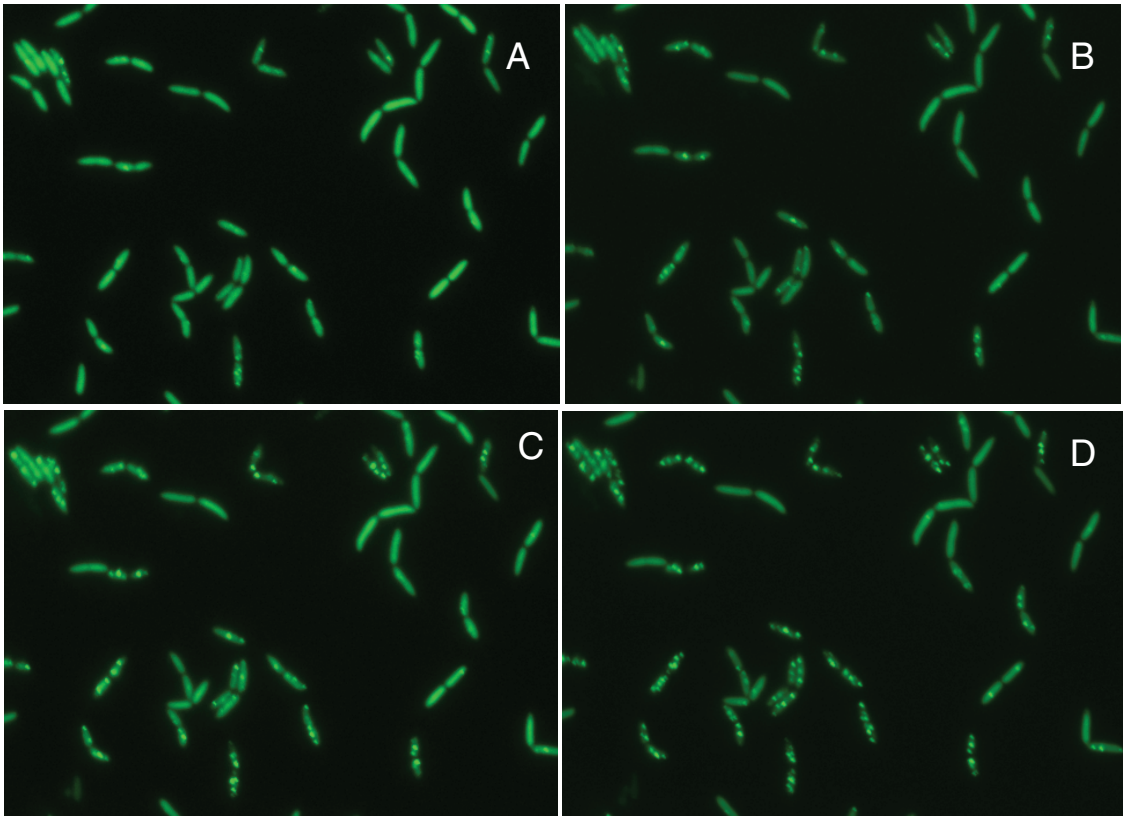


FIG. 3. Time course of infection with bacteriophage Φ6. Cells of LM4622 were infected with Φ6 and visualized at 34 min (A), 45 min (B), 60 min (C), and 75 min (D) after infection.

protein were bound to infecting particles and whether they remained bound to these particles *in vivo*. The present study with GFP fusions has enabled us to demonstrate that the host protein is bound to incoming virions and that the association continues until lysis. Most fluorescent particles remain throughout infection; some disappear, and some new ones appear. This is consistent with the observation that about 20% of the incoming virion cores seem to recycle into mature virions but most of the infecting particles do not end up in mature virions again (16). The punctate pattern did not appear immediately upon infection; under the conditions of these experiments, it seemed to take approximately 30 min for the infecting particles to show fluorescence. This was seen with a phage that has a nonsense mutation in gene 2, resulting in no new functional particles being formed. We suggest that the delay is due to the time for protein P8 to be removed from the incoming nucleocapsids. Experiments to follow the fate of P8 have shown that it is removed from infecting particles and almost completely digested by 45 min after infection (16). The achievement of maximum levels of dot formation takes about 60 min postinfection and seems to involve the covering of a small number of new particles that does not increase further until the onset of lysis beginning at 120 min after infection. This might involve the binding of YajQ to incoming particles that have lost P8 and to a small number of newly filled particles.

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