

Host Range of Chlamydiaphages ϕ CPAR39 and Chp3

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The host range of ϕ CPAR39 is limited to four *Chlamydomphila* species: *C. abortus*, *C. caviae*, *C. pecorum*, and *C. pneumoniae*. Chp3 (a newly discovered bacteriophage isolated from *C. pecorum*) shares three of these hosts (*C. abortus*, *C. caviae*, and *C. pecorum*) but can additionally infect *Chlamydomphila felis*. The ability to support replication was directly correlated with the binding properties of the respective bacteriophages with their host species. Binding studies also show that ϕ CPAR39 and Chp3 use different host receptors to infect the same host cells: cell binding is sensitive to proteinase K treatment, confirming that the chlamydiaphage receptors are proteinaceous in nature.

Chlamydiae have an obligate intracellular developmental cycle that alternates between the infectious elementary body and the replicative form, the reticulate body (RB). Five bacteriophages have been isolated from the chlamydiae. The first chlamydial bacteriophage to be characterized (13) was found by thin-section transmission electron microscopy (EM) of avian *Chlamydomphila psittaci* RBs (14). Although samples containing Chp1 were later lost, a second bacteriophage (ϕ CPG1) (7) was found to infect *Chlamydomphila caviae* (8) and a third bacteriophage, Chp2, was isolated from *Chlamydomphila abortus* (10). The *Chlamydomphila pneumoniae* strain AR39 genome-sequencing project revealed the presence of a double-stranded DNA extrachromosomal element (11), which was subsequently shown to be the replicative form of bacteriophage ϕ CPAR39 (4). Recently Chp3 was isolated from *Chlamydomphila pecorum* (6) and was found to share 97.1% nucleotide sequence identity with Chp2. Chlamydiaphages ϕ CPAR39 and ϕ CPG1 also show 97.1% identity, whereas the two groups (Chp2–Chp3 and ϕ CPAR39– ϕ CPG1) share 93.3% identity. All the chlamydiaphages share similar features; they are small icosahedral T = 1 particles containing circular, single-stranded DNA genomes, and molecular characterization showed that they belong to the virus family *Microviridae* (9–11, 17).

Recently we described the host range of Chp2 and showed that Chp2 was unable to infect *C. caviae* or *C. pneumoniae*. It was therefore of interest to investigate the host range of the very closely related Chp3 and the slightly more diverse bacteriophages ϕ CPAR39 and ϕ CPG1, which were isolated from host chlamydiae resistant to Chp2. In our work, monolayers of BGMK (Buffalo green monkey kidney) or HEp2 cells in 25-cm² flasks were infected by centrifugation at 1,000 \times g for 1 h in medium containing cycloheximide (1 μ g/ml) and gentamicin (25 μ g/ml) with *C. pecorum* bearing Chp3 or *C. pneumoniae* (11) bearing ϕ CPAR39. At 72 h postinfection the culture medium was replaced with a small volume of phosphate-buffered saline (PBS), and the flasks were frozen at -70°C . One hun-

dred flasks of phage-infected chlamydiae were prepared, stored frozen, and then processed as a single batch. The flasks were frozen and thawed three times to lyse the chlamydial RBs and release phage. Any remaining monolayer that had not detached after this procedure was scraped off. The suspension was centrifuged at 2,000 \times g for 15 min to sediment the cell debris. The supernatant was passed through a 0.45- μ m-pore-size filter followed by a 0.22- μ m-pore-size filter. The filtrate was centrifuged at 100,000 \times g in a Beckman SW28 rotor for 3 h, and the resultant pellet was washed with PBS and centrifuged at 80,000 \times g for 40 min. The pellet was finally suspended in PBS, vortexed with glass beads, and stored at -70°C as a partially purified bacteriophage preparation. A standard inoculum of Chp3 or ϕ CPAR39 shown to be capable of infecting >99% of *C. pecorum* or *C. pneumoniae* IOL 207 by inclusion staining was mixed with each of a range of chlamydial species for 30 min at room temperature. The mixture was diluted in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, cycloheximide (1 μ g/ml), and gentamicin (25 μ g/ml), and BGMK cells cultured on coverslips were infected by centrifugation at 1,000 \times g for 1 h. A set of coverslips were prepared in parallel with unchallenged chlamydiae. When inclusions were clearly visible (from 48 to 72 h postinfection), monolayers were fixed in ice-cold methanol. The susceptibility of chlamydiae to infection with ϕ CPAR39 or Chp3 was monitored by fluorescence microscopy by using a phage-specific monoclonal antibody (MAb 55) as previously described (4). The tropism of ϕ CPAR39 and Chp3 was tested by experimental infection of all chlamydial species and is summarized in Table 1. ϕ CPAR39 produces a characteristic inclusion staining pattern when infecting *C. pneumoniae*, showing the formation of enlarged RBs (4). This phenomenon was observed in two independent isolates of *C. pneumoniae* when infected with ϕ CPAR39. By contrast, infection of *C. caviae*, *C. pecorum*, and *C. abortus* by ϕ CPAR39 gives whole inclusion staining (data not shown), indicative of complete RB lysis, and *C. caviae* was susceptible to Chp3 infection. In the case of *C. caviae* infection with Chp3, lysis occurred rapidly. This led us to recheck the effects of Chp2 on *C. caviae*, which we had previously scored as negative. Repeated infections of *C. caviae* with

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TABLE 1. Host range and binding properties of ϕ CPAR39 and Chp3^a

Genus and species	Strain	Source or reference	Chlamydial host species	ϕ CPAR39		Chp3	
				Sensitivity	Binding	Sensitivity	Binding
<i>Chlamydophila abortus</i>	A22	16	Ovine	S	B	S	B
<i>Chlamydophila psittaci</i>	Cal 10	5	Avian	—	No	—	No
<i>Chlamydophila pecorum</i>	E58	VR-628	Bovine	S	B	S	B
<i>Chlamydophila felis</i>	FP	2	Feline	—	No	S	B
<i>Chlamydophila pneumoniae</i>	CWL 029	VR-1310	Human	S	B	—	No
	TW 183	VR-2282	Human	S	ND	—	ND
<i>Chlamydophila caviae</i>	GPIC	VR-813	Human	S	B	S	B
				—		—	
<i>Chlamydia trachomatis</i>	L1/440/LN	15	Human	—	No	—	No
<i>Chlamydia suis</i>	DC-6	K. Sachse	Porcine	—	ND	—	ND
<i>Chlamydia muridarum</i>	MoPn	VR-123	Murine	—	ND	—	ND

^a Abbreviations and symbols: S, sensitive to infection; —, not sensitive to infection; B, binding; No, no binding; ND, not done.

Chp2 showed that it is highly susceptible to Chp2 infection. We speculate that previous results were originally scored negative for infection by Chp2 because the life cycle of Chp2 in *C. caviae* is so rapid that it lyses RBs early in the developmental cycle before inclusions fully develop, making their detection difficult.

Previously it had been shown that host restriction of Chp2 was mediated through the ability of this bacteriophage to bind to a chlamydial surface protein-based receptor (4). In the present host challenge study we monitored for bacteriophage replication by immunofluorescence assay and showed that ϕ CPAR39 is unable to infect *C. felis* and Chp3 is unable to infect *C. pneumoniae*. Quantitative cell binding assays (Fig. 1) confirmed that ϕ CPAR39 cannot bind to *C. felis* and Chp3 cannot bind to *C. pneumoniae* but that ϕ CPAR39 binds to *C. pneumoniae* and Chp3 binds to *C. felis*, demonstrating that host restriction is due solely to receptor recognition on a susceptible host. These studies were extended to include an example of each chlamydial species (Table 1), and the results confirmed that the ability of ϕ CPAR39 or Chp3 to bind to elementary bodies (EBs) correlated directly with their ability to replicate and cause infection.

We have shown that the Chp2 receptor is a protease-sensi-

tive component of the chlamydial outer membrane (4). To test whether the receptor(s) for ϕ CPAR39 and Chp3 was also protease sensitive, EBs of both *C. felis* and *C. pneumoniae* were treated with proteinase K and then binding assays were performed as previously described (4). These analyses (Fig. 1) indicate that treatment of EBs with protease completely eliminates binding to susceptible host bacteria by phages ϕ CPAR39 and Chp3.

Chlamydiae do not encode an external scaffold or major spike protein (10). Alignment of the structural proteins of Chp2 and Chp3 with those of ϕ CPAR39 showed very little variation except in the coat protein VP1, which has an area of significant divergence between amino acids 216 and 299. The related microviruses ϕ MH2K and SpV4 also have this distinctive region of sequence variation within their coat proteins (1). For SpV4, cryoimage reconstruction shows a large insertion loop that forms spikes at the threefold axis of symmetry (3). These spikes are thought to be formed by trimerization of the insertion loop (IN5) at each threefold axis of symmetry of the capsid and are a relic either of the external scaffolding protein or of a major spike protein (1). It has been speculated that in chlamydiae similar insertion loops are the viral antireceptors (12). Our observations are consistent with this pro-

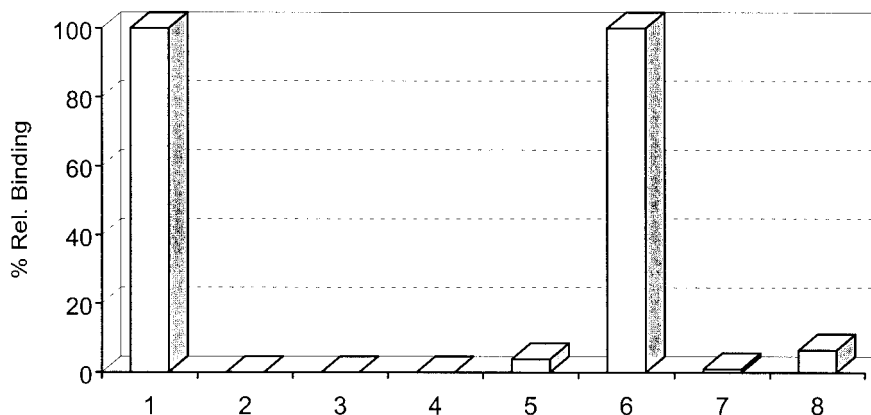


FIG. 1. Binding of Chp3 and ϕ CPAR39 with susceptible and nonsusceptible hosts. Shown are relative binding of *C. felis* EBs by Chp3 (column 1) and ϕ CPAR39 (column 2) and the effects on such binding by Chp3 (column 3) and ϕ CPAR39 (column 4) following treatment of EBs with proteinase K. Columns 5 to 8 show relative binding to *C. pneumoniae* EBs by Chp3 (column 5) and ϕ CPAR39 (column 6) and the effects on such binding by Chp3 (column 7) and ϕ CPAR39 (column 8) following treatment of EBs with proteinase K.

posal, as Chp2 and Chp3 have an identical cell tropism which is different from that of ϕ CPAR39 and the predicted IN5 equivalent sequences of both Chp2 and Chp3 are highly conserved, with only two amino acid changes from each other, whereas the IN5 region of ϕ CPAR39 is 15 amino acids shorter and highly divergent (12). The simplest explanation is that Chp2-Chp3 and ϕ CPAR39 bind to different outer membrane protein receptors via the IN5 domain. This hypothesis is supported by the observation that saturation of the susceptible host *C. abortus* by Chp2 and Chp3 does not prevent binding with ϕ CPAR39 (data not shown). Thus, ϕ CPAR39 and Chp3 infect both *C. abortus* and *C. pecorum*, because their respective individual receptors are both present in these species.

Studies of bacteriophages that infect chlamydiae are of great interest, because they may provide the foundation for a genetic transfer system. Surface proteins, such as bacteriophage receptors, may also be involved in host-pathogen interactions, and while many chlamydial outer envelope proteins have been identified, many more remain to be discovered and the functions of most remain obscure. The observation that chlamydiae have different but overlapping cell tropisms is an important and significant discovery because it affords the opportunity to create recombinant chlamydiae by coinfection using host species of chlamydiae susceptible to both groups of chlamydiae. Future work will focus on producing the necessary monoclonal antibodies that will allow the selection and isolation of recombinant chlamydiae. These will then be used to map antireceptor binding domains to explore in fine detail the role of extracellular factors on cell tropism.

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