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Construction of a Transducing Virus from Double-Stranded RNA Bacteriophage φ6: Establishment of Carrier States in Host Cells

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Bacteriophage φ6 contains three double-stranded RNA (dsRNA) genomic segments. We have constructed a plasmid that contains a cDNA copy of the middle (M) segment, with a gene for kanamycin resistance (*kan*) inserted into the *PstI* site. A transcript of this cDNA was incorporated in vitro into procapsids along with natural transcripts of the S and L segments. The procapsids were coated with nucleocapsid surface protein P8 and transfected into *Pseudomonas syringae* pv. phaseolicola. The resulting infectious virus, φ6 K1, was found to contain an M segment that was 1.2 kbp larger than the normal 4.1 kbp. K1 formed small, turbid plaques, and its genome was unstable. Preparations of K1 contained from about 0.1 to 10% large, clear-plaque forms of the virus which were usually missing the *kan* gene, and in some cases, the resulting segment M was smaller than its normal size. Cells picked from lawns of host cells infected with K1 yielded colonies that were resistant to kanamycin (Kan). These colonies could be passaged on kanamycin-containing medium. The cells were found to contain large amounts of dsRNA corresponding to the viral genomic segments. Some strains continued to produce viable phage, while others lost this ability. One strain completely lost the small genomic segment S. Approximately 1 in 10,000 infected cells acquired the carrier state with the original phage isolate K1. However, we isolated a viral mutant that was able to induce the carrier state in 10 to 20% of the infected cells. The ability to use drug resistance as a test for the carrier state makes this system very useful for the study of the mechanisms of induction of persistent infections.

Bacteriophage φ6 infects the plant pathogen *Pseudomonas syringae* pv. phaseolicola HB10Y (31). Three separate pieces of double-stranded RNA (dsRNA) compose its genome, which is located inside a polyhedral nucleocapsid (NC) (27). The NC has RNA polymerase activity (22) and is covered by a lipid-containing membrane (31). The assembly pathway involves the formation of a polyhedral procapsid, which is then filled with one copy each of the three pieces of dsRNA per virion (7, 17). These filled procapsids are then covered with a shell of protein P8 to become NCs, which are subsequently enveloped within the lipid-containing membrane (14).

We have developed an in vitro genomic packaging system that utilizes single-stranded RNA (ssRNA) obtained from the in vitro transcription of viral NCs or RNA transcripts of plasmids carrying cDNA copies of the viral genomic segments. The RNA is packaged by procapsids formed in *Escherichia coli* cells that are expressing the cloned genes for φ6 procapsid proteins P1, P2, P4, and P7. In this way, we have demonstrated that a recombinant transcript of segment M that contains an engineered 4-bp deletion can be incorporated into the genome of viable phage (21).

In this report, we show that a transcript containing an insertion of the kanamycin resistance (*kan*) gene can be incorporated into viable virus and that this virus can establish a carrier state in host cells. The carrier state (CS) cultures show resistance to kanamycin. A carrier state for φ6 has been described previously (6, 23) as appearing among phage-resistant colonies of the host of φ6.

Carrier states and persistent infections have been described for many viruses that are ordinarily lytic or cytopathic. The carrier states of reovirus, a dsRNA virus, have been well studied. It has been shown that mutations in both the host cells (1) and the virus (5) can affect the frequency of production of the carrier state.

MATERIALS AND METHODS

Bacterial strains, phage, and plasmids. *P. syringae* pv. phaseolicola HB10Y (31) was used as the host for φ6 lysate preparations, for phage titration, and for the determination of infectious centers in the NC infection experiments (Table 1). The receptorless φ6-resistant derivative of HB10Y, HBMPO.16 (24), was used for the preparation of spheroplasts that can be infected by φ6 NC. Bacteriophage φ6 (31) was used for the preparation of phage NCs used in ssRNA production and as the source of the NC coat protein P8. The expression plasmid pLM450 (12) containing φ6 L segment cDNA was used for the production of phage procapsids in *E. coli* JM109. JM109 was the host for the propagation of recombinant plasmids.

Preparation of φ6 ssRNA. φ6 ssRNA was synthesized by an in vitro transcription reaction modified from that of Emori et al. (8), using NCs prepared as described by Mindich et al. (18). The individual ssRNA species were isolated from the original transcription mixture by rate zonal sucrose gradient centrifugation (5 to 20% sucrose, 50 mM Tris-Cl [pH 7.5], 300 mM NaCl; Beckman SW41 rotor, 17 h, 4°C, 21,000 rpm). The gradients were fractionated, and the ssRNA zones were identified in composite gels (30). The peaks were collected, concentrated by ethanol precipitation, and dissolved in water.

Construction of plasmid pLM672. The construction of plasmid pLM656, which yields a transcript identical to the viral M ssRNA species, has been described before (21). The

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TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Remarks	Reference or source
Bacteria		
P. syringae pv. phaseolicola		
HB10Y	Host of φ6	31
MPO.16	Resistant to φ6	24
P. pseudoalcaligenes ERA	Alternative host of φ6	16
E. coli JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ⁻ Δ(*lac-proAB*) [F' *traD36 proAB lacI*^{q}ZΔM15]	34
Phages		
φ6	Original wild-type strain	31
φ6*hls*	Host range mutant of φ6	16
sus59	Amber mutation in gene 8	17
K1	φ6 with *kan* insert	This study
K14	Very turbid derivative of K1, high transduction efficiency	This study
Plasmids		
pT7T3 19U	*amp*, P_{T7}, P_{T3}, *lacZ'*	Pharmacia
pUC-4K	*amp lacZ'*, *kan* cartridge in *Pst*I site	32
pLM450	*tet*; contains cDNA copy of segment L; expresses P1, P2, P4, and P7	12
pLM656	*amp*, P_{T7}; exact copy of segment M in pT7T3 19U	This study
pLM672	*amp*, P_{T7}; *kan* gene in *Pst*I site of pLM656	This study

vector is plasmid pT7T3 19U (Pharmacia). This plasmid has promoters for both T7 and T3 polymerases as well as the *lacZ'* element of pUC19 (34). It also has the replication origin of phage f1. The *kan* gene from plasmid pUC-4K (32) was excised with *Pst*I and inserted into the *Pst*I site of the cDNA copy of segment M in pLM656 (Fig. 1). The resulting plasmid was designated pLM672. The insert adds 1,241 bp to the genomic segment. The *Pst*I site is in the noncoding region of the segment, and therefore no genes are disrupted. The insert begins with the *Pst*I site, then 12 G's, followed by the sequence AAAGCCAC, starting at position 1052 of Oka et al. (20). The insert terminates with GACCTCAGCG, which ends at position 2263 of Oka et al. (20), then 12 C's, and the *Pst*I site. We have sequenced the junctions to confirm the construction.

Preparation of procapsids. φ6 procapsids were prepared as described by Gottlieb et al. (11) using the expression plasmid pLM450 (12) propagated in *E. coli* JM109. The procapsid preparation contained 400 μg of protein per ml, as determined by the Bradford assay (4).

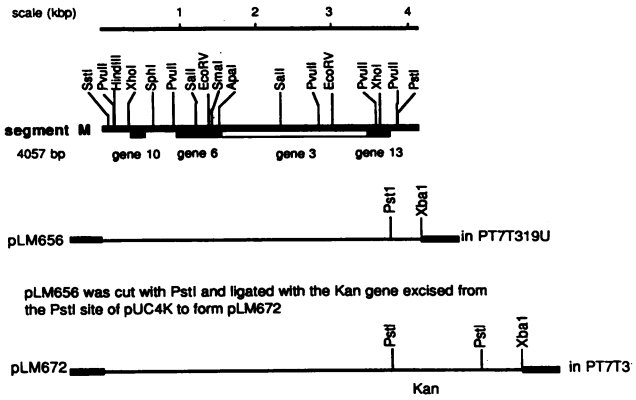


FIG. 1. Construction of plasmid pLM672. The cDNA copy of genomic segment M is shown with its restriction sites. An exact copy of this segment was constructed in vector pT7T3 19U so that the first nucleotide of the T7 RNA polymerase transcript would be the first nucleotide of the positive-sense strand of segment M (21). Cutting the plasmid with *Xba*I and treating with mung bean nuclease before transcription resulted in the formation of the same 3' end in the transcript as in the positive-sense strand of M. The kanamycin resistance gene of plasmid pUC-4K was excised with *Pst*I and ligated into the *Pst*I site of pLM656.

Polymerase reaction conditions. The φ6 RNA polymerase reaction mixes contained 50 mM Tris-Cl (pH 8.2), 3 mM $MgCl_2$, 100 mM ammonium acetate, 20 mM NaCl, 5 mM KCl, 5 mM dithiothreitol, 0.1 mM disodium EDTA, 1 mM each ATP, GTP, CTP, and UTP, 5% polyethylene glycol 4000, and 40 mg of Macaloid per ml (9). To each 25-μl reaction mixture, 500 to 750 ng of φ6 ssRNA and 1.4 μg of procapsid protein were added. The amount of the synthetic M transcript was 5 to 10 μg. The reaction mix was incubated at 28°C for 75 min.

Assembly of coat protein. Purified protein P8 was assembled on the packaged procapsids in 31.5-μl mixtures as described previously (21).

NC infection. HBMPO.16 cells were rendered competent for φ6 NC infection as described previously (19). To each 120-μl aliquot of cells, 20 μl of each coat protein assembly mixture was added, and the infection was allowed to proceed in 35-μl droplets on Millipore VSWP02500 filters on LB plates overlaid with LB top agar, 3% lactose, and 20 mM potassium phosphate, pH 7.2, at room temperature for 60 min. Thereafter, the cells were washed once with 1 ml of LKSB buffer (19) and resuspended in 100 μl of the same buffer, and infectious center titers were determined on HB10Y lawns.

DNA sequence analysis. Sequence analysis was performed by the dideoxy chain termination method (26) with bacteriophage T7 DNA polymerase (Sequenase; U.S. Biochemicals). The synthetic deoxyoligonucleotides used as primers were 5'-CGCCTCAAGCAACTGCATTG (OLM55), which anneals to a sequence starting 50 bp downstream from the *Pst*I sites, and 5'-CGAGTGCCGACCGCTCGCGG (OLM59), which anneals to a sequence beginning 220 bp upstream from the *Pst*I sites of pLM672 (Fig. 1).

Analysis of protein and RNA synthesis. Cultures of CS cells were grown in M8 medium, treated with rifampin (200 μg/ml), and labeled with tritiated leucine or uridine as described previously (29). Cells were collected and dissolved in cracking buffer and analyzed on 15.5% polyacrylamide

gels for proteins (15) or on 2% polyacrylamide composite gels for RNA (28, 30).

Electron microscopy. Colonies were removed from agar plates containing LB medium with 40 μg of kanamycin per ml, suspended in phosphate buffer, and fixed with 3% glutaraldehyde for 25 min at room temperature. The fixed cells were then processed and sectioned as described previously (3).

Marker rescue test. The ability of a particular CS culture to donate a genomic segment to a superinfecting phage was determined by spotting dilutions of phage containing nonsense mutations in specific genes on the surface of a plate containing a lawn of the CS culture. The nonsense mutants used carried *sus351* (gene 1, L), *sus507* (gene 6, M), and *sus297h591* (gene 12, S) (13, 18). This test could only be carried out on HB10Y strains that were not producing phage because the endogenously produced phage resulted in too many plaques per plate to see the rescue. In the case of the *Pseudomonas pseudoalcaligenes* ERA strains, the phage that was produced endogenously did not plate on ERA and therefore did not interfere with this test. The ERA strains, however, could not be used for the rescue of segment M because K1 lacks the *h1* mutation necessary for plating on ERA. The *h1* mutation is a change in gene 3 that alters the pilus-binding specificity of protein P3 (16).

Determination of transduction frequency. Small turbid plaques of K1 were picked from a lawn of HB10Y. These were suspended in LB medium and filtered through polycarbonate membrane filters (0.2 μm; Bio-Rad Laboratories). Approximately 10^6 PFU were added to 10^8 cells and incubated for 15 min at 23°C. The cells were pelleted and resuspended in 0.2 ml of LB. An aliquot was plated for the determination of infectious centers, and another aliquot was spread on LB plates containing kanamycin (40 μg/ml). Alternatively, cultures of CS cells were grown overnight in LB with kanamycin (40 μg/ml). The cells were removed by centrifugation and then by filtration. The filtrates were assayed as described above.

Sedimentation analysis of filled procapsids. ERA strain 12 is a derivative of ERA that contains procapsids which are replicating genomic segment L and segment M carrying the *kan* cartridge. It is missing segment S. Strain 12 was grown in LB with kanamycin and harvested in the logarithmic growth phase. Cells were suspended in buffer and then processed in a French pressure cell as described previously except that the buffer contained 0.3 M NaCl (12). The lysate was centrifuged at 10,000 rpm for 10 min to remove debris, applied to a 10 to 30% sucrose gradient containing 0.3 M NaCl, centrifuged, and analyzed (12).

A culture of HB10Y was infected with mutant *sus59*, which has an amber mutation in gene 8 and produces procapsids (17). Filled procapsids were isolated by lysing cells with lysozyme (25). The lysis buffer contained 0.3 M NaCl instead of 1.5 M in the original protocol. The lysates were centrifuged for 10 min at 10,000 rpm to remove debris and applied to gradients as described above. Gradients were run with individual samples of particles from the *sus59* mutant or ERA12 or mixtures of the two, in order to ascertain whether the particles that contained segments L and M sedimented differently than those containing all three segments.

Preparation of RNA from cells. Cells were grown overnight in LB medium with kanamycin. The cultures were collected by centrifugation, lysed, and treated by the method of Godson and Vapnek (10). The nucleic acid preparations were then treated with RNase-free DNase I (Boehringer 776 785).

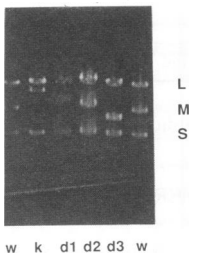


FIG. 2. dsRNA extracted from virions. dsRNA was extracted from purified virions. Lanes: Wild-type ϕ6 (w); K1 (k); clear-plaque derivatives of K1 (d1, d2, and d3). The size of segment M in sample k is interpolated to be 5.3 kbp, which is 1.2 kbp larger than that in wild-type virus.

RESULTS

Isolation of ϕ6 K1. A mixture of zone-purified segments S and L and the T7 RNA polymerase transcript of pLM672 was incubated with procapsids isolated from *E. coli* JM109 as described in Materials and Methods. The packaging mixture was then incubated with purified protein P8 to coat the filled procapsids. Spheroplasts of strain HBMPO.16 were incubated with this mixture. The infected spheroplasts were plated on a lawn of HB10Y. A single plaque was found and picked. This strain was designated ϕ6 K1. This plaque yielded phage that were heterogeneous in plaque morphology. Most of the plaques were small and turbid, but some were large and clear. The clear plaques remained clear; however, the turbid-plaque formers continued to show heterogeneity. Although it was difficult to prepare pure stocks of the turbid-plaque formers, several preparations were made by taking care to adjust the amount and purity of the phage placed on each plate. The RNA extracted from the turbid-plaquing phage contained normal-sized genome segments L and S; however, the size of segment M was 5.3 kbp instead of the normal 4.1 kbp (Fig. 2, lane k). This increase was consistent with its containing the *kan* gene of plasmid pLM672. The clear plaques yielded phage preparations with normal segments L and S and variable sizes of segment M (Fig. 2, lanes d1, d2, and d3).

Establishment of CS cultures. Approximately 10% of the phage-resistant colonies that appear on confluent lawns of ϕ6-infected *P. syringae* pv. phaseolicola are able to release phage during the growth of cultures (6, 23). This carrier state is fairly stable and persists for a number of plate passages.

We tested for the appearance of colonies that were resistant to kanamycin after infection with K1. The turbid-plaque phages yielded such colonies. The original screening was done at 25 μg of kanamycin per ml. The colonies that appeared were tested for growth at higher drug concentrations, and it was found that they could grow at drug concentrations as high as 1 mg/ml. The clear-plaque phages did not yield kanamycin-resistant colonies.

RNA extracted from the kanamycin-resistant cultures showed a gel migration pattern characteristic of the parental recombinant phage. The estimated molecular weight of segment M was consistent with its carrying the *kan* gene.

Ten independently formed resistant colonies were passaged weekly and tested for the production of viable phage. All were capable of producing phage for several passages.

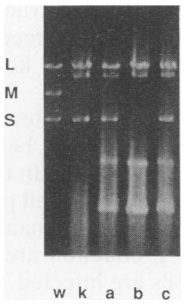


FIG. 3. dsRNA extracted from CS cells. RNA was extracted from cultures growing in LB medium with kanamycin. ERA strain 3, which produces virus, is shown in lane a; ERA strain 12, which does not produce virus, is shown in lane b; and ERA strain 11, which also does not produce virus, is shown in lane c. Lane w contains RNA isolated from wild-type virus, and lane k contains RNA isolated from K1 virions.

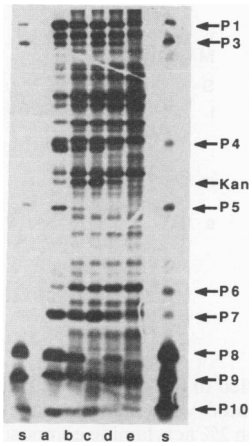


FIG. 4. Proteins labeled in the presence of rifampin. CS cultures of strain ERA were treated with rifampin, labeled with [^{3}H]leucine, lysed, and analyzed on 15.5% polyacrylamide gels (15). Lane s, normal cells of HB10Y infected with φ6; lane a, ERA strain 3; lane b, ERA strain 11; lane c, ERA strain 12; lane d, ERA strain 18; lane e, normal ERA cells. The positions of φ6 proteins are shown on the side of the autoradiogram. The position of the *kan* gene product is designated by Kan.

The phages that were produced were capable of forming plaques on the parental CS strains. We found that wild-type phage could also plate on these cultures but that the plating efficiency was low. Plating was complicated, however, by the plaques formed by the endogenously formed phage particles. After three to eight passages, 3 of the 10 strains lost the ability to produce phage. These strains maintained their resistance to kanamycin, and two continued to replicate viral RNA, as observed by gel analysis. It was possible to test the ability of the CS cells to rescue nonsense mutants of φ6. This marker rescue test involved the exchange of whole genomic segments between the CS cells and the infecting nonsense-mutant phage. In this way, we could ascertain whether the individual genomic segments were still functional in virus infection. We found that the cells that produced virus had all three functional segments. Two of the three nonproducers were defective in segment M. In addition, segment S in one of them had become smaller than normal, but it was found to be functional in the rescue test. The remaining nonproducer, missing the three genomic segments, appeared to have been mutated chromosomally to gain resistance to kanamycin and was no longer in the carrier state.

P. pseudoalcaligenes ERA is an alternative host for φ6. The virus requires a host range mutation, *hl*, in gene 3, which specifies the attachment protein P3, in order to adsorb to this host at high efficiency (16). When exposed to the recombinant phage φ6 K1 at a high multiplicity of infection, ERA cells that had acquired resistance to kanamycin could be isolated. Thirty independent colonies were isolated and passaged weekly. Initially, 21 strains were able to produce phage that plated on HB10Y, but these viruses were not able to plate on the producing strains of ERA or on uninfected ERA. This was presumably due to the lack of the *hl* mutation. The original infections leading to the carrier state were apparently accomplished by virus that infected the cells despite the lack of the host range mutation. Most of the ERA CS strains, both phage producing and nonproducing, were resistant to at least 40 μg of kanamycin per ml and contained viral RNA of the expected migration pattern (Fig. 3). In the rescue test, some of the nonproducers were defective in segment L, some in M, and some in S. The defect in L is of special interest in that these cells were still capable of replicating the viral RNA. In one case, segment S was completely lost from the carrier strain (Fig. 3, lane b). Probing with an oligonucleotide complementary to the ends of the three segments gave no evidence of the persistence of a smaller deleted segment S (data not shown). Analysis of the sedimentation behavior of filled procapsids from this strain showed that the particles had less mass than normal filled procapsids containing all three segments (data not shown). These results indicate that particles can replicate segments L and M independently of S.

The synthesis of viral proteins was demonstrated in the CS cells by labeling with radioactive leucine in the presence of rifampin (Fig. 4). The presence of late proteins such as P8 and P3 was clear. In the case in which segment S was missing, none of the proteins encoded by S were labeled (Fig. 4, lane c). The product of the *kan* gene, aminoglycoside 3′-phosphotransferase, has a molecular mass of 30.7 kDa (20). It can be seen in the lysates of the CS cells (Fig. 4). dsRNA was effectively labeled in the presence of rifampin (Fig. 5), consistent with the notion that the viral RNA polymerase is active in the CS cells. Electron micrographs of thin sections of the CS cells showed viral particles in approximately the same density as seen in infected cells (Fig. 6). Both fully mature, enveloped and unenveloped particles can be seen. The HB10Y cells (Fig. 6B) are not as densely packed with phage as found previously for some of the CS cells (23). In the ERA cells in which segment S was missing, the particles were not enveloped (Fig. 6D); however, the cells of ERA strain 11, which carried all three genomic segments, also showed many particles that lacked an envelope.

The carrier state is rather unstable but can be propagated in the absence of drug selection. When Kan cells of ERA are plated on agar without drug, most of the colonies show a variable loss of drug resistance upon retesting. Since the growth rate of the CS cells is close to that of normal cells, we estimate that the rate of loss of the carrier state must be on the order of 1 to 10% per division. The cultures that have reverted from the carrier state show a return to normal sensitivity to φ6*hls*. The carrier state in HB10Y is somewhat

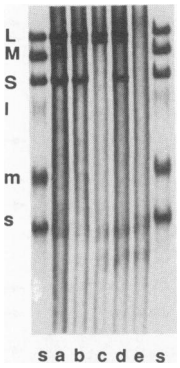


FIG. 5. RNA labeled in the presence of rifampin. CS cultures of strain ERA were treated with rifampin, labeled with [^{3}H]uridine, lysed, and analyzed on 2% acrylamide composite gels (30). Lanes: a, ERA strain 3; b, ERA strain 11; c, ERA strain 12; d, ERA strain 18; e, normal ERA cells. Lanes s contain ^{32}P-labeled dsRNA and ssRNA from an in vitro transcription of ϕ6 virions. L, M, and S are the dsRNA genomic segments, and l, m, and s are their respective ssRNA transcripts.

more stable than in ERA, but we estimate a probability of loss of about 1% per division.

Small plaques of ϕ6 K1, the original transducing phage, yielded CS colonies in approximately 1 of 10,000 infectious centers formed at a low multiplicity of infection. CS cells liberate infectious phage into the medium with titers of about 10^6 PFU/ml. The frequency of induction of the carrier state was higher in the CS culture supernatant fluids than found for isolated plaques of the original virus. When CS culture supernatant fluids were used, it was commonly found that 0.1 to 1% of the new infectious centers were Kan. In one case, the frequency of formation of Kan colonies was 10 to 20%. This phage was designated K14. Its plaques were even more turbid than the original transducing phage K1. These mutants were used to prepare high-titer phage stocks that continued to show a high frequency of Kan transduction. The mutation conferring the high-level transducing ability has been tentatively mapped to genomic segment L, which encodes the replicase proteins of the virion.

DISCUSSION

We show in this study that ϕ6 can carry extra genetic material in its genome. The *kan* gene is expressed in infected cells. A carrier state can be established in the infected cells and can lead to a condition in which the resistance gene is replicated and expressed but virus is no longer produced. As long as kanamycin selection is applied, this system operates as an episome, much like the natural dsRNA episomal system in *Saccharomyces cerevisiae* (33).

The virus carrying the *kan* cartridge is genetically unstable. It is not yet clear whether the instability is due to the problems of carrying an extra 1.2 kbp in a genome of 13.5 kbp or whether it is due to the structure of the extra RNA. The *kan* gene of pUC-4K is bounded by a *Pst*I site and 12 G residues on the 5′ side and 12 C residues and a *Pst*I site on the 3′ side. The resulting hairpin structure might be a cause of the genetic instability. The segment M RNA in the CS cells is rather stable as long as kanamycin selection is maintained.

When considering the carrier state, it is always necessary to differentiate between what has been called the "treadmill" state and a true carrier state. In the treadmill state, the virus is growing in a subset of the cell population and the rest are uninfected. A steady state is maintained in which both viable cells and virus production are present. In the true carrier state, all the cells are infected, and the replication of the viral genome is compatible with cell viability. This is certainly the case for the true carrier state that we describe in this article. In order for the cells to grow in the presence of kanamycin, they must contain the viral genome and express the *kan* gene. Any subset of the population that is not carrying the phage genome would be sensitive to the drug.

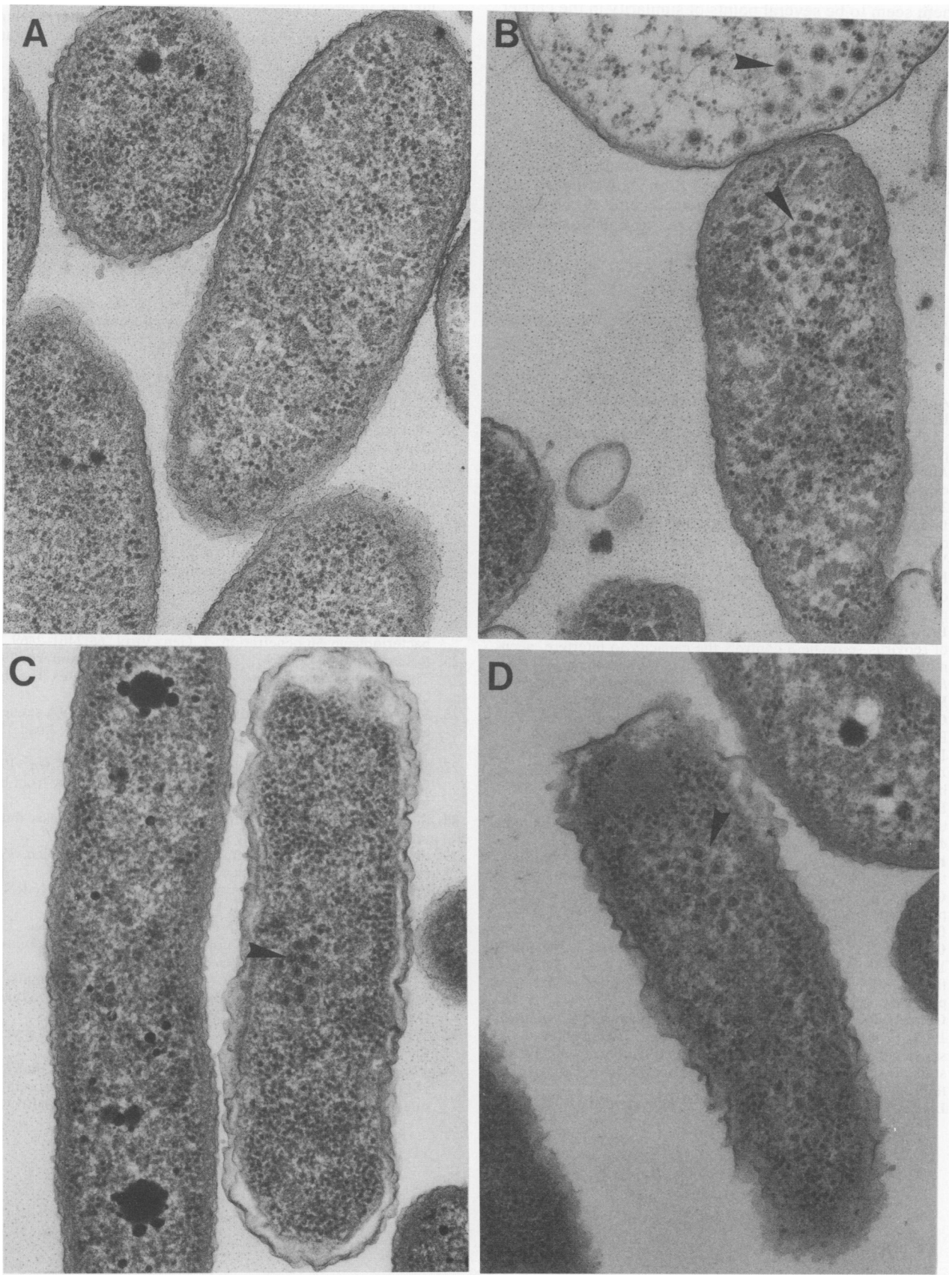
The CS cells appear to be somewhat less sensitive to ϕ6 than normal cells, but they are not completely resistant. Wild-type phage can form plaques on CS cells, but the efficiency of plating is between 1 and 10% of that of normal cells. Romantschuk and Bamford (23) demonstrated that cultures of HB10Y derived from persistently infected cultures had increased tolerance for lysozyme even after being cured of ϕ6. These cultures also showed much greater resistance to ϕ6 than we have found in the present study. It is possible that the high levels of phage resistance found in the earlier studies were the result of selection during the continued production of phage. We found that CS cells of strain ERA that had lost the viral system showed normal sensitivity to ϕ6. This suggests that a host mutation is not necessary for the establishment or maintenance of the carrier state. It is still possible, however, that host mutations may be able to increase the frequency of carrier state formation.

If the host cells in the carrier state are not mutated with respect to phage resistance, then the reason for the relative resistance in the carrier state should be phage mutations or the appearance of a special physiological state. The mechanisms involved in phage-mediated lysis are still poorly understood, but it would seem that anything that would interfere with cell lysis could facilitate the carrier state. In addition, ϕ6 infection leads to a shutdown of host protein synthesis (29). This condition must be averted in CS cells as well.

It is not clear whether infectious virus is being produced in all cells or whether the genome that is replicated in the carrier state is completely intact. It might be that there are inactivating mutations in the genome that is present in the CS cells and that the infectivity of the virus which is produced in these cultures is due to new mutations needed to attain an infectious state.

Our isolation of K14, a phage mutant that is able to form the carrier state 1,000 times more frequently than normal virus, supports the notion that a prior viral mutation is necessary for the formation of the carrier state but that the virus does not need to mutate again in order to escape from the carrier state.

FIG. 6. Electron micrographs of thin sections of CS cells. (A) Normal HB10Y; (B) HB10Y strain J, which produces infectious virions; (C) ERA strain 11, which does not produce live phage but contains all three genomic segments; (D) ERA strain 12, which is missing genomic segment S. The arrows point to intracellular virus particles. Most of the particles do not show a viral membrane with the exception of those at the top of panel B in the cell that is lysing. Magnification, ×65,000.


A
B
C
D

There seem to be several points of similarity in the carrier state that we have observed and that seen with reovirus. Reovirus is capable of establishing persistent infections. It has been shown that mutations in the S4 gene of reovirus facilitate the establishment of persistent infection, while those in the S1 gene play a role in the maintenance of the effect (2). It has also been shown that mutations in the L2 gene affect the frequency of persistent infections (5), perhaps because they increase the frequency of mutations in other genes, such as S1 and S4. It has also been shown that L cells derived from cured, persistently infected cultures can establish a new persistent infection with reovirus more easily than normal L cell lines (1).

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