

Mutator Bacteriophage D108 and Its DNA: an Electron Microscopic Characterization

GURNAM S. GILL,[†] RICHARD C. HULL,[‡] AND ROY CURTISS III*

Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294

Three types of phage particles were observed on CsCl step gradients when D108 was purified from lysates prepared by induction of a prophage. These particle types were identified to be the mature phage, tailless DNA-filled heads, and a form of nucleoprotein aggregates. The nucleoprotein aggregates banded at a density (ρ) of >1.6 . DNA molecules isolated from mature phage particles were (38.305 ± 1.226) kilobases (kb) in length. Denaturation and renaturation of D108 DNA resulted in the formation of linear double-stranded molecules with variable-length single-stranded tails at one end. About 30% of the annealed molecules also carried an internal nonhomology, which was shown to be the region called the G-loop in Mu and P1 DNAs. Following the notation used for different regions of denatured, annealed Mu DNA, we measured the lengths of the equivalent D108 DNA regions to be as α -D108 = (32.178 ± 1.370) kb; G-D108 = (3.07 ± 0.382) kb; β -D108 = (2.291 ± 0.306) kb; SE-D108 = (0.966 ± 0.433) kb. Formation of D108:Mu heteroduplexes disclosed the presence of five nonhomologies, two of which were partial. One of the partial heterologies was in the G-loop region. The largest nonhomology, (1.393 ± 0.185) kb in size, was near the *c* end (immunity region) and probably spans the *c* and the *ner* genes of Mu. β -D108 was shown to carry a (0.556 ± 0.097) -kb insertion close to its right end. A short 100-base-pair region appeared to have been conserved at the ends of D108 and Mu. Occasionally, a 50- to 100-base-pair-long unpaired region was also observed at the left end of D108:Mu heteroduplexes. These sequences were presumably of bacterial DNA. Taken together, our results complement and extend our earlier genetic studies which established that D108 was a mutator phage heteroimmune to Mu with a host range different from Mu's.

Bacteriophage D108 was isolated by Mise as a generalized transducing phage different from P1 (22). It transduces chromosomal markers at frequencies between 10^{-8} to 10^{-6} per phage. Data on cotransduction frequencies of nearby markers by D108 indicate that its DNA complement is roughly half that of P1 (22). No physical characterization of D108 DNA has been reported, however.

It has been concluded from genetic and physical studies on the mature and replicative DNA molecules of the other generalized transducing phages like P1, P22, T1, and Mu, that they all mature their DNAs by the headful-type mechanism (3, 16, 19, 25, 27). With the exception of Mu DNA, mature DNAs of the above-mentioned phages have been shown to have terminal redundancies and permutations of their nucleotide sequences (9, 16, 24). Mature Mu DNA molecules (DNA molecules isolated from phage

particles), instead of showing a terminal redundancy, carry host chromosomal DNA at their termini (1, 6). The presence of terminal redundancy and permutations of the nucleotide sequences in phage DNA is thought to be a consequence of cleaving and packaging sequential headfuls of DNA during maturation from a concatemeric replicative form of DNA. If the capacity of the head is somewhat larger than the genome size, taking sequential headfuls of DNA from the concatemer will result in the mature molecules being terminally redundant and cyclically permuted. However, if further constraints, such as initiation or maturation at a specific site combined with directional processing by the headful or limiting the length of the concatemer or both, are imposed on the packaging machinery, the resulting mature molecules will have a limited number of permutations, as is found to be the case with P22, T1, and Mu DNAs. Whereas P22 and T1 DNAs have about 10 and 3 permutations of their DNA sequences, respectively, Mu DNA shows only one (3, 9, 27). The lack of permutations and terminal redundancy

[†] Present address: Division of Infectious Diseases, The Upjohn Company, Kalamazoo, MI 49001.

[‡] Present address: Department of Microbiology, School of Medicine, University of Washington, Seattle, WA 98195.

in Mu DNA is probably due to the peculiar mode of its replication. The replicated intermediates of Mu DNA are always found to be covalently linked to the host chromosomal DNA (18, 28).

We wanted to know what category D108 DNA would fit into, i.e., whether its DNA was permuted or terminally redundant or both, etc. The simplest way to ascertain the presence of permutations and terminal redundancy in mature phage DNA molecules is to denature and anneal the molecules and then examine the annealed structures under an electron microscope. If the molecules were permuted and terminally redundant, denaturation and annealing would result in the formation of circular and linear molecules with variable-length, single-stranded tails. When D108 DNA was subjected to such a treatment, no circular molecules were observed. All the annealed molecules were linear, and virtually all of them had two variable-length, single-stranded tails at one end. About 30% of the molecules also showed an internal nonhomology. Such structures are known to occur in denatured, annealed Mu DNA (5). Thus, it was obvious that either D108 was a reisolation of Mu or the phage was closely related to Mu. In this report we show that D108 is, indeed, closely related to Mu. Heteroduplexes formed between D108 and Mu DNAs revealed five full or partial nonhomologies. Two of the nonhomologous regions, one near the immunity end of Mu and the second in the G-loop area, correlated well with the genetic differences shown previously by us to exist between D108 and Mu, namely, the heteroimmunity and different host ranges (13).

MATERIALS AND METHODS

Bacteriophages, bacterial strains, and media. *Mu*cts62, a heat-inducible derivative of Mu, was kindly provided by M. Howe. A heat-inducible derivative of D108, called D108 *cts10* was isolated in this laboratory and has been described previously (13). χ 289 is a prototroph and was used as a host for preparation of phage stocks. All bacterial cultures were grown in L broth which contains (per liter) 5 g of NaCl, 5 g of yeast extract, 10 g of tryptone, and 0.1% glucose. The pH of the medium was adjusted with NaOH to be between 7.0 and 7.4.

Preparation of phage lysates. A 200-ml culture of χ 289 (*Mu*cts62) was grown at 30°C by aeration to a cell density of 4×10^8 cells/ml. The prophage was induced by shifting the culture to 42°C, followed by vigorous shaking for 15 min. After the prophage induction, the culture was transferred to another shaker maintained at 37°C, where it was aerated vigorously until cell lysis. Cell debris was removed by spinning the lysate at 12,000 rpm in a refrigerated Sorvall centrifuge. Phage particles were concentrated by sedimentation for 1.5 h at 19,000 rpm in an SS-34 rotor of the Sorvall centrifuge. The supernatant fluid was dis-

carded, and the phage pellets were suspended by gentle shaking in the cold in 0.5 ml of a buffer containing 10 mM Tris, 10 mM NaCl, and 10 mM MgCl₂, pH 8.0.

D108 *cts10* was prepared from a 1-liter culture of χ 289 (D108 *cts10*). Four 250-ml cultures in 2-liter flasks were grown at 30°C to a cell density of 3×10^8 to 4×10^8 cells/ml. The prophage was induced by transferring the flasks to 38°C, followed by vigorous aeration until the cultures cleared. D108 *cts10* is very heat inducible and is efficiently induced at 37 to 38°C, thus precluding the step of shifting cultures to 42°C. After removal of cell debris with a slow-speed spin, phage particles were precipitated by polyethylene glycol as described previously (29). Precipitated particles were suspended in 5 ml of buffer containing 10 mM Tris, 10 mM MgCl₂, pH 8.0.

Purification of phage and isolation of DNA. Phages were purified by two CsCl step gradients as described by Thomas and Davis (26). In the first run, 1-ml steps of CsCl solutions of $\rho = 1.6, 1.5,$ and $1.4,$ respectively, were gently layered on top of one another in a nitrocellulose tube of an SW50.1 rotor. Phage lysate was layered on top of the final step ($\rho = 1.4$), and the tubes were spun at 30,000 rpm for 1 h in the Beckman SW50.1 rotor. The phage bands were extracted from the side of the tube by puncturing with a 23-gauge needle. In the second gradient, the phage particles were floated upwards as follows. Phage band extracted from the first gradient was mixed with an equal volume of saturated CsCl solution and was transferred to a nitrocellulose tube. Again, steps of CsCl solutions of $\rho = 1.6, 1.5,$ and $1.4,$ respectively, were layered on the phage suspension and centrifuged as described in the first step gradient. In some cases where two closely spaced phage bands from a step gradient were to be separated well, the bands extracted from the first gradient were mixed with 2 ml of a CsCl solution of $\rho = 1.5$ and were spun to equilibrium in an SW50.1 rotor at 30,000 rpm.

For DNA isolation, phage particles were first dialyzed into TNE buffer (10 mM Tris, 10 mM NaCl, 1 mM EDTA, pH 8.0), followed by three extractions with equal volumes of water-saturated phenol. Residual phenol was removed by extraction with two volumes of chloroform. Subsequently, traces of phenol or chloroform or both were removed by extensive dialysis against TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Electron microscopy. Phage particles were visualized under the electron microscope by staining with uranyl acetate. Phage particles were first adsorbed to a carbon-coated copper grid by putting a 10- μ l drop of the phage-containing solution on the grid. After 1 min, the drop was removed by touching it with the edge of a piece of filter paper. The adsorbed particles were stained with a 2% aqueous uranyl acetate solution by touching the grid to a 20- μ l drop of the stain solution for 1 min. Excess stain was removed with a piece of filter paper, and the grid was air dried.

All the heteroduplexes were formed and mounted for electron microscopy by the methods described by Davis et al. (7). Double-stranded (ds) and single-stranded (ss) ϕ X174 DNAs were used as internal length standards. Photographed molecules were projected onto a screen, traced, and measured by a graph-

ics calculator (Numonics Corp., Lansdale, Pa.). All ss DNA lengths were corrected by using the internal length standards, ϕ X174 ds and ϕ X174 ss.

RESULTS

Purification of phage particles. When D108 and Mu phage particles from a 200-ml culture purified on CsCl step gradients, invariably two phage bands were observed between the junction of the CsCl steps of $\rho = 1.5$ and $\rho = 1.4$. The phage bands were called the L (light) and the H (heavy) bands, respectively. The H band usually contained about 20 to 25% of the material observed in the L band. However, when D108 particles from a 1-liter culture were purified on a step gradient, three phage bands were observed. Whereas two of the bands were in the same position as the L and the H bands as observed previously, the third band, called HH, was located in the lower half of the first step ($\rho = 1.6$). Each of the bands, i.e., L, H, and HH, was removed separately by puncturing the tube from the side. After further purification on a second step gradient and an equilibrium density gradient of appropriate initial density ($\rho = 1.5$ for the L and H particles; $\rho = 1.6$ for the HH particles), each of the particle preparations was titrated on χ 289 for the presence of PFUs. Only the material from the L band had PFUs. When dilutions of particles from the H and HH bands were plated so as to get 10^3 to 10^4 plaques on χ 289 lawns, no plaques were observed. However, if similar numbers of particles (in a 10- μ l volume) from the heavier bands were spotted on the lawn, the spots were cleared. Visualization of the particles under the electron microscope disclosed that the particles in the L band were mature phages, the H band contained tailless DNA-filled heads, and the HH band contained some aberrant structures, probably some sort of nucleoprotein aggregates (Fig. 1). Inspection of the complete particles indicates that there may be at least three tail fibers attached to a base plate. The tail has a contractile sheath, and a necklike region is also seen between the upper end of the tail sheath and the head. No effort was made to measure the dimensions of different components of the phage particles. DNA molecules isolated from the L and the H band particles were of the same size. Not enough material was available to examine the size and form of the DNA molecules in the HH particles.

Characterization of D108 DNA. D108 DNA molecules released from purified phage particles by phenol extraction were mounted for electron microscopy, photographed, and measured as described above. Using the length of ϕ X174 ds DNA of 5.386 kilobases (kb) as the

standard, the average length of D108 DNA (for 40 molecules) was measured to be (38.305 ± 1.226) kb (11). If the average molecular weight of a base pair (bp) is taken as 660, the molecular weight of D108 DNA will be $(25.281 \pm 0.809) \times 10^6$.

To find out whether D108 DNA sequences were terminally redundant or permuted or both, we examined with the electron microscope the DNA structures formed after annealing a denatured D108 DNA preparation. No circular molecules were detected. On the contrary, all renatured molecules were linear and always showed variable-length, single-stranded tails at one end. About 30% of the molecules also carried an internal nonhomology, which was located towards the end of the molecules with the ss tails. Some of these bubbles also showed some intramolecular sequence homology (Fig. 2). A cross section of different types of structures seen in annealed preparations of D108 DNA is shown in Fig. 2, and the size measurements on each region are listed in Table 1.

Structures similar to the ones mentioned above have been previously observed and characterized in denatured and annealed Mu DNA (3-5). The internal nonhomologous region in Mu DNA has been named the G-loop, and the end with the terminal ss tails has been called the split end (SE). These tails have been shown to be bacterial DNA covalently linked to the phage DNA (6). We have adopted the same terminology for different segments of D108 DNA (Fig. 3 and Table 1). The region to the left of the G-loop is called α , whereas the one between the G-loop and the SE is called β . This is illustrated in Fig. 3, and the sizes of the respective regions of D108 are given in Table 1. The G-loop of Mu DNA is an invertible region which has small (~50 bp) inverted repeats at its end (4, 5). When Mu is grown by induction, inversion of the G-loop has been shown to occur in 30 to 50% of the molecules. The inversion occurs in only 1% of the molecules if the phage is grown by infection of a host (5). We observed similar rates of inversion of the G-loop in D108, which were dependent on the mode of phage growth. This was accomplished as follows. Phage particles were purified: (i) from a lysate made by induction of a culture grown from a single colony of χ 289 (D108 *cts10*), and (ii) from a lysate made by infection of χ 289 by D108 *cts10* at a multiplicity of infection of 2. DNA from each phage preparation was denatured, annealed, and examined under an electron microscope. In each case, 100 intact molecules were examined. In the phage preparation made by induction, 31 of the molecules displayed an internal nonhomology closer

to the SE, whereas only three such molecules were found in the preparation made from phage grown by infection. The size of the G-loop, (3.070 ± 0.382) kb, in D108 heteroduplex molecules is exactly the same as reported for Mu (3-5). By inference, it was assumed that the terminal ss tails at the SE of D108 heteroduplexes were pieces of bacterial DNA. To compare the sizes of the mature D108 and Mu DNA molecules, Mu DNA was measured under the same conditions described for D108 DNA. The average length of Mu DNA (from 37 molecules) was (38.768 ± 1.780) kb, which is equivalent to a

molecular weight of $(25.586 \pm 1.175) \times 10^6$. These values are close to the measured values of D108 DNA. In the light of these observations and our previous report that D108 is a mutator phage, it was clear that the two phages might be closely related to each other (13).

D108:Mu heteroduplexes. The extent of relatedness and divergence between D108 and Mu DNAs was assessed by forming heteroduplexes between the two phage DNAs. Some of the heteroduplex molecules are shown in Fig. 4. There are five nonhomologous regions, two of which are probably partial heterologies (or par-

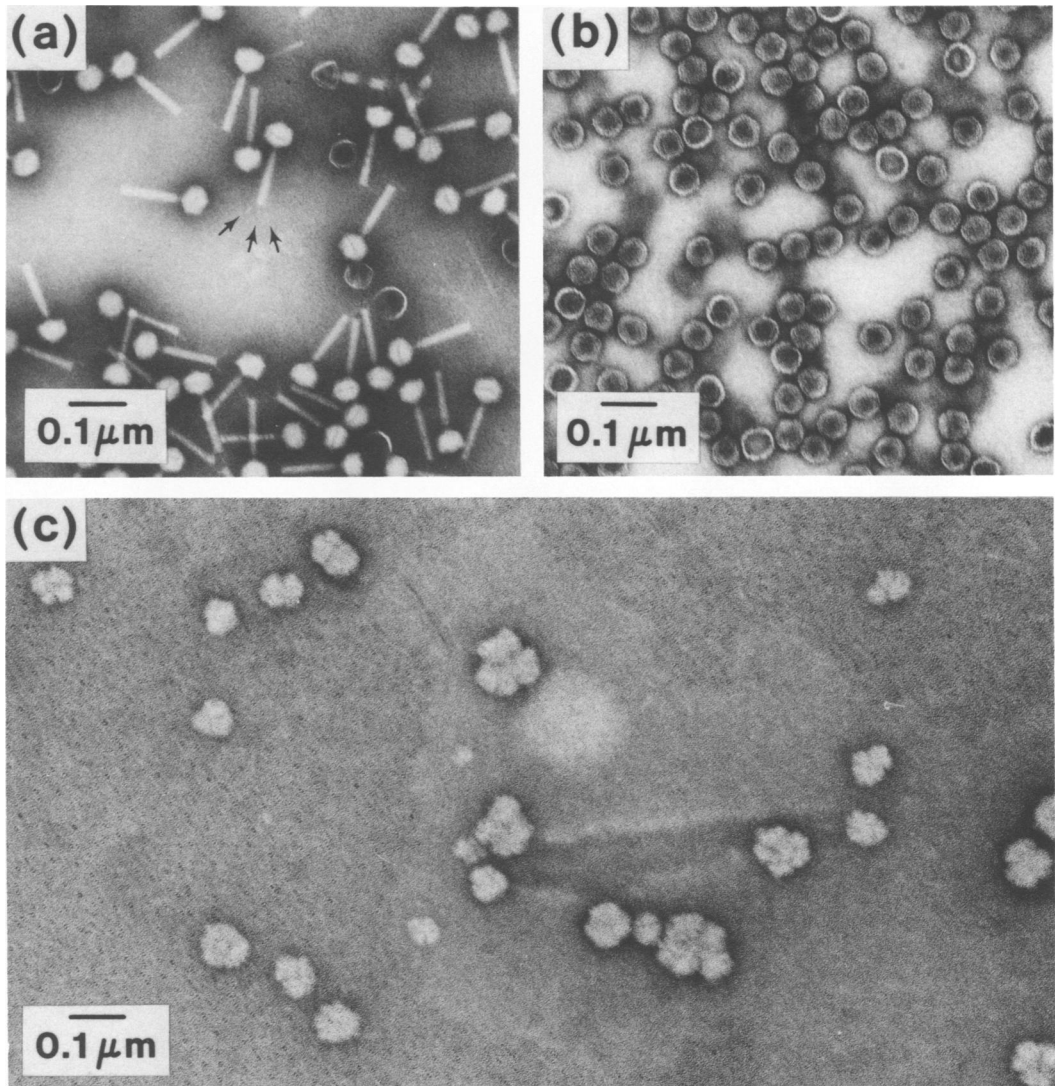
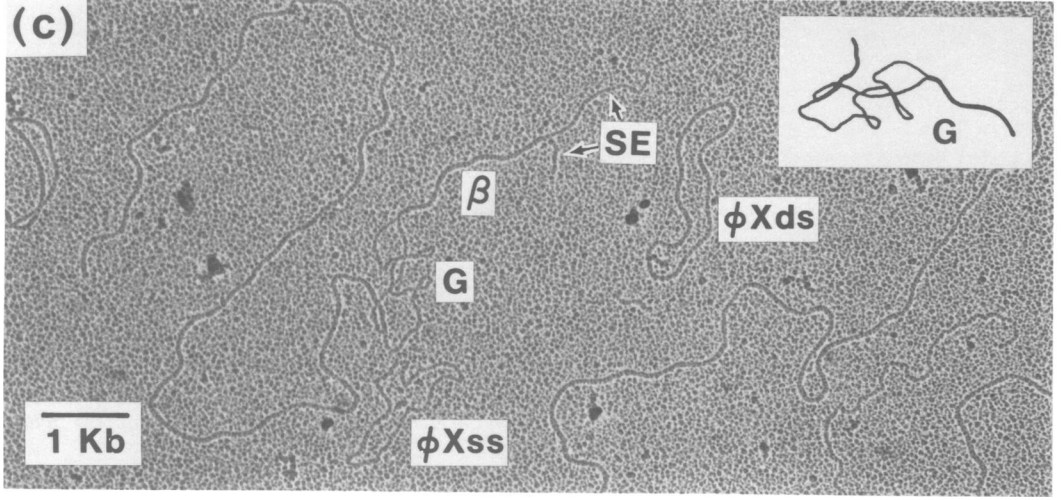
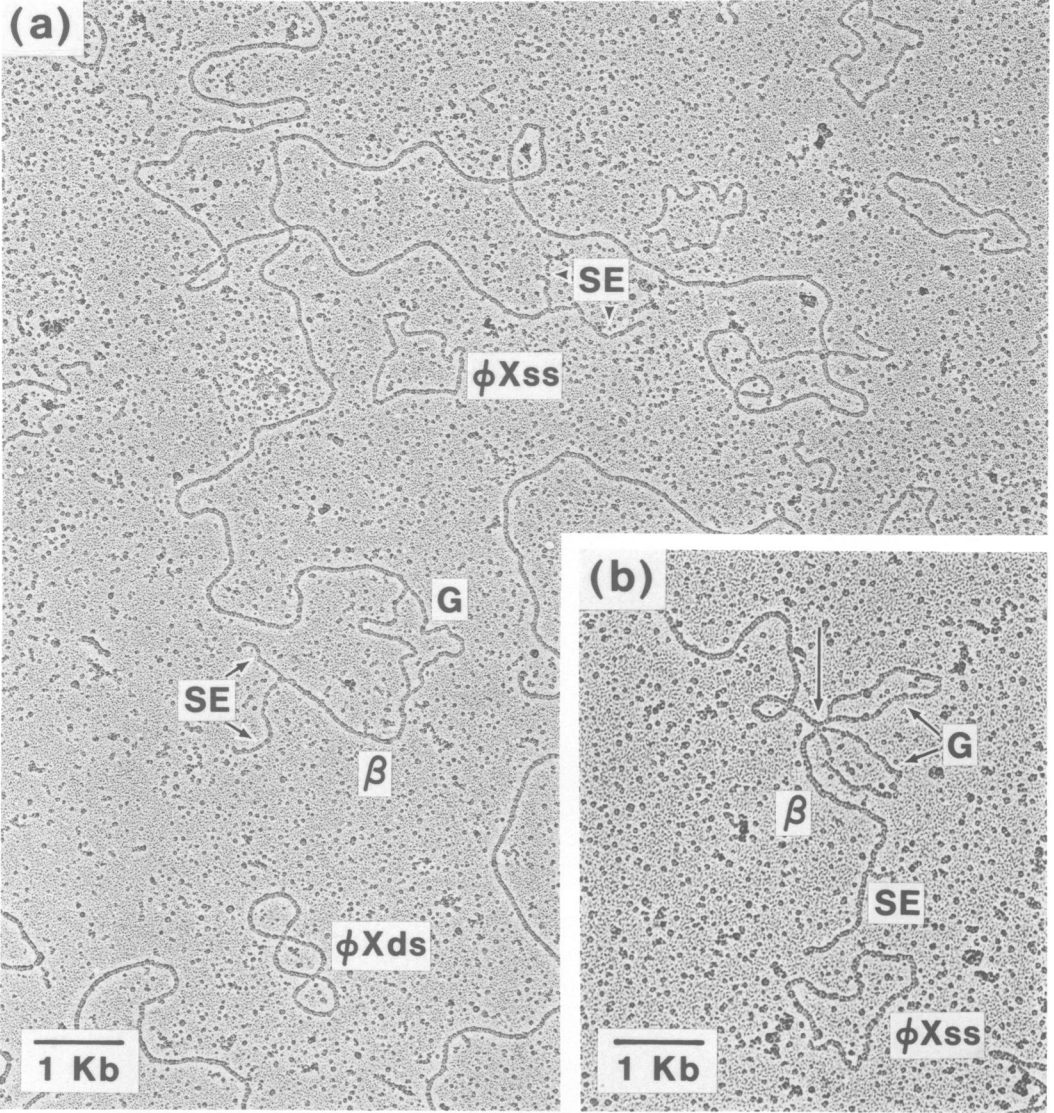


FIG. 1. D108 particle types observed during purification of phage on CsCl step gradients: (a) particles in the L band, (b) particles in the H band, and (c) particles in the HH band. The mature particles appear to have three tail fibers, which are marked by three arrows in (a).



tial homologies) and one of which is an insertion. One of the partially homologous regions (labeled G partial) is in the G-loop area, and its position in different molecules appears to shift from one end of the G-loop to another (data not shown) and is, of course, seen in the molecules which have the G-loop in the same orientation (Fig. 4). This shift in the position of the partial homology in the G-loop can be explained on the basis of the inversion of the G-loops in both D108 and Mu. The second partial homology is the region marked D in the molecules (Fig. 4 and Fig. 5). One of the reasons we think these are partial homologies is that they are not seen in all the heteroduplex molecules when the DNA is spread for electron microscopy from hyperphases of 40, 50, and 60% formamide. However, they appear more often as the formamide concentration is increased from 40 to 60%. One could argue that these regions are adenine-thymine rich and hence denatured in high formamide concentrations. We think this is unlikely because they were never observed when D108 DNA that was denatured and then renatured was spread from a 60% formamide hyperphase.

There are two nonhomologous regions very close to the ends of the heteroduplex molecules. The nonhomology marked B is towards the left end (c end) and is the largest, (1.393 ± 0.185) kb in size. The one called J (J1 and J2 due to unequal arms) is close to the SE and is an insertion of (0.556 ± 0.098) kb. Two small ds regions marked A [(0.114 ± 0.037) kb] and K [(0.107 ± 0.039) kb] near the ends should also be noted (Fig. 4 and Fig. 5). Both these regions, i.e., A and K, were found to be stable even in 60%

formamide, implying that the sequences in these regions have been conserved during evolution of these two phages. Size measurements on different regions of the D108:Mu heteroduplexes are depicted in Fig. 5.

DISCUSSION

When phage particles were purified from D108 lysates made from thermally induced $\chi 289$ (D108 *cts10*), three types of particles were observed on CsCl gradients. These particle types consisted of the mature phage (L particles), the tailless DNA-filled heads (the H particles), and some form of nucleoprotein complexes (the HH particles) (Fig. 1). The size of the DNA molecules isolated from the L and the H particles was the same, whereas the size and the type of the DNA molecules in the HH particles could not be determined due to lack of material. DNA in the H and the HH particles is infectious as judged by the spotting test described above.

Denaturation and renaturation of D108 DNA leads to the formation of structures similar to those observed in denatured, annealed Mu DNA (5). The size of the G-loop in D108 DNA was measured to be 3 kb (Table 1). A number of published reports give the size of the G-loop in Mu DNA to be 3 kb also (3-5). In D108 DNA, the G-loop has displayed similar structural features as in Mu, i.e., the variable-length, partial intrastrand homologies ranging in size from 77 to 425 bp and small inverted repeats at the ends (Fig. 2). Formation of D108:Mu heteroduplexes indicates that the G-loop sequences in Mu and D108 are very similar, if not identical because, when in the same orientation, they show high degree of homology under mild denaturing conditions (40% formamide). However, examination of the heteroduplex molecules in 50 to 60% formamide reveals partially homologous sequences (Fig. 4), implying that at some point in time the G-loop sequences in D108 and Mu have diverged. Recently, it has been reported that two

TABLE 1. Length measurements on denatured and annealed D108 molecules^a

Region	No. of molecules measured	Mean length ^b (kb)
α	14	$(32.178 \pm 1.3707 \text{ SD}^b)$
G	88	(3.070 ± 0.382)
β	42	(2.291 ± 0.306)
ss Tails ^c	75	(0.966 ± 0.433)
D108 DNA	40	(38.305 ± 1.226)
Mu DNA	37	(38.768 ± 1.780)

^a All lengths were measured relative to the length of $\phi X174$ ds. The length of $\phi X174$ ds DNA was taken as 5,386 bp or (5.386) kb. Measurements on native D108 and Mu DNAs are also included.

^b SD, Standard deviation.

^c These are variable-length ss tails seen at the SE of the denatured and annealed molecules (see Fig. 3).



FIG. 3. Drawing showing names of different regions of the molecules in D108 self-heteroduplexes, particularly those molecules which carried an internal nonhomology called the G-loop. Nomenclature used is the same as applied to Mu DNA heteroduplexes.

FIG. 2. Electron micrographs showing the different molecule types seen when D108 DNA is denatured and annealed. In (a) two molecules are seen, one of which has an internal nonhomology, whereas both show ss tails at one end. Molecules in (b) and (c) show the internal structure of the G-loop. In (b) an arrow points to a small stem in one of the arms of the G-loop.

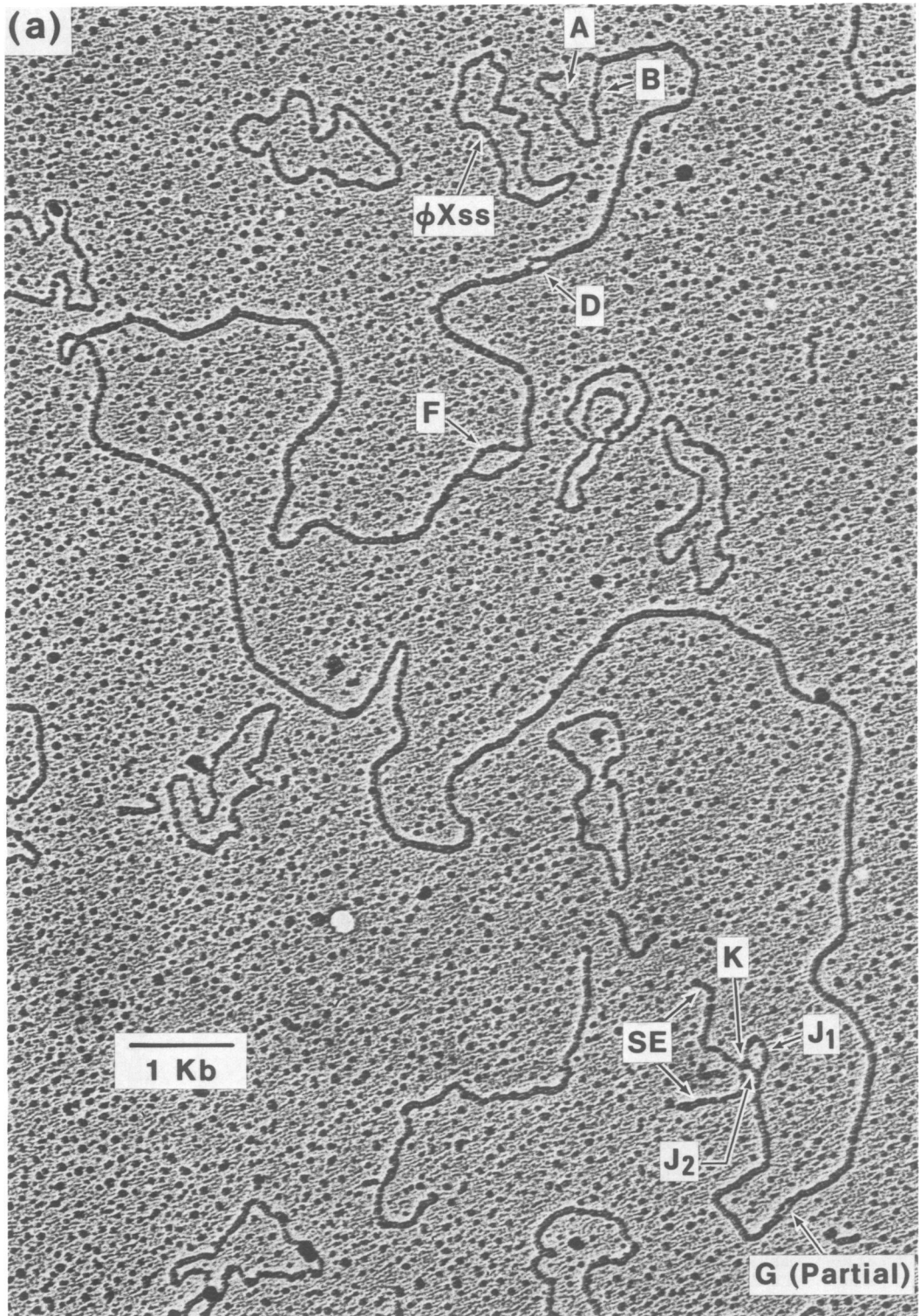
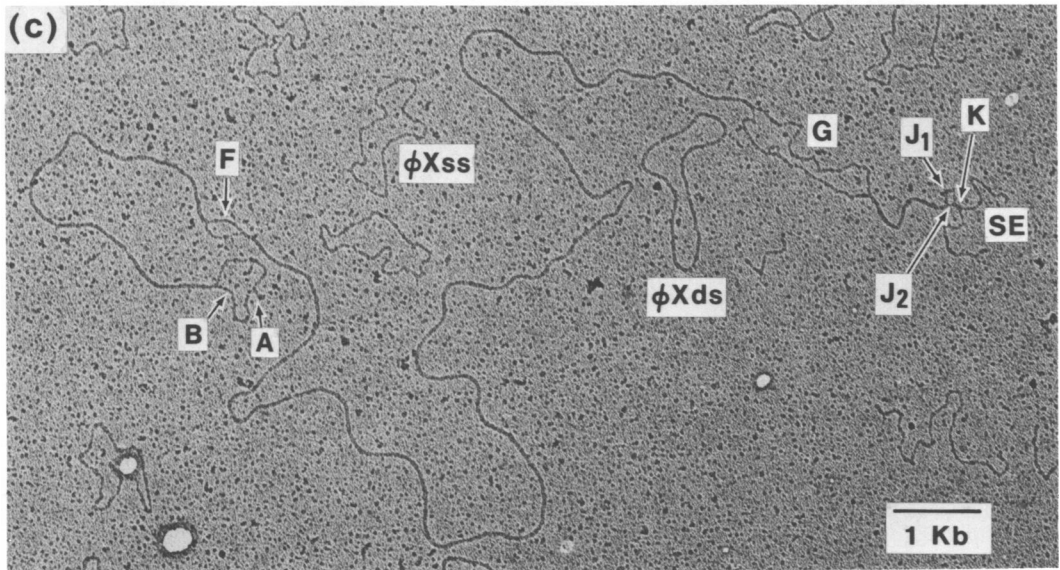
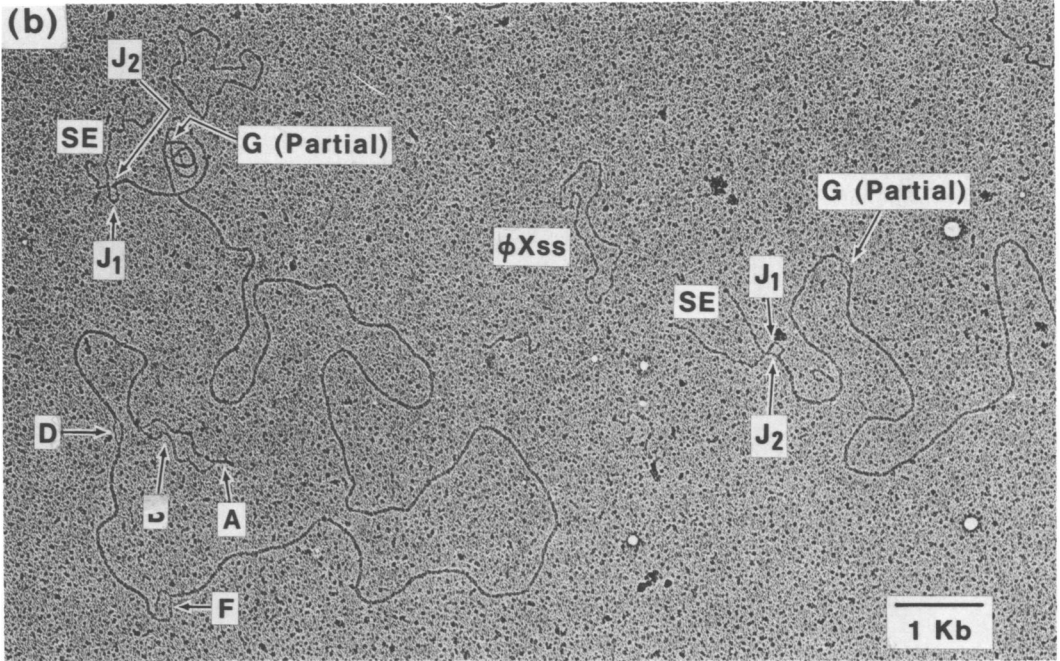


FIG. 4. Electron micrographs showing a sampling of D108:Mu heteroduplexes. Molecules in (a) and (b) show all the nonhomologies, including the two partial ones, i.e., D and G (partial). In these molecules, the G-loops of D108 and Mu are in the same orientation. Also, in (b) there might be two short tails to the left of A, although this cannot be said with absolute certainty. In the molecule in (c), the G-loops are in opposite orientation, and D is missing. All these molecules were spread from a 60% formamide hyperphase.



of the Mu genes, the S and U genes, which may determine the host range of the phage, are located in the G-loop (12, 23). Our earlier studies showed that bacterial strains resistant to Mu are generally sensitive to D108 (13). Thus, the divergence observed in the G-loop sequences of D108 and Mu correlates well with the observed differences in their host range. The G-loop region of Mu and D108 has been identified in P1 and P7 DNAs also (4, 15). It has been reported that cells resistant to Mu are often sensitive to P1 (4). Although the G-loop regions of Mu and P1 appeared completely homologous under mild conditions, they might reveal differences similar to those observed in D108 and Mu G-loops if spread under more denaturing conditions (4). Since P1 has a much broader host range than Mu, it is not implied that the host range of P1 is solely determined by the genes located in the G-loop.

Heteroduplexes formed between D108 and Mu DNAs revealed a 50- to 100-bp-long homologous region located at the ends of the molecules (marked A and K in Fig. 5) that has been conserved in both phages. It is known that the ends of Mu DNA are essential for its integration in the host chromosome or into plasmid DNA (8). Direct DNA sequencing of the ends of Mu DNA has established the presence of short, partially inverted repeats, and potential stem-loop structures which might be formed through interaction between the ends of Mu during its integration or transposition into the target DNA have been proposed (14, 15). The stability of A and K under fairly harsh denaturing conditions (60% formamide) points to the importance of these regions in both phages.

To the right of homology A, we come across the largest nonhomology, labeled B, approximately 1.4 kb in length (Fig. 5). The left-most gene of Mu, the *c* gene, codes for a 24,000-molecular-weight immunity or repressor protein (17, 20, 21). Such a protein could be coded by ~700 bp of Mu DNA. By infecting minicells with

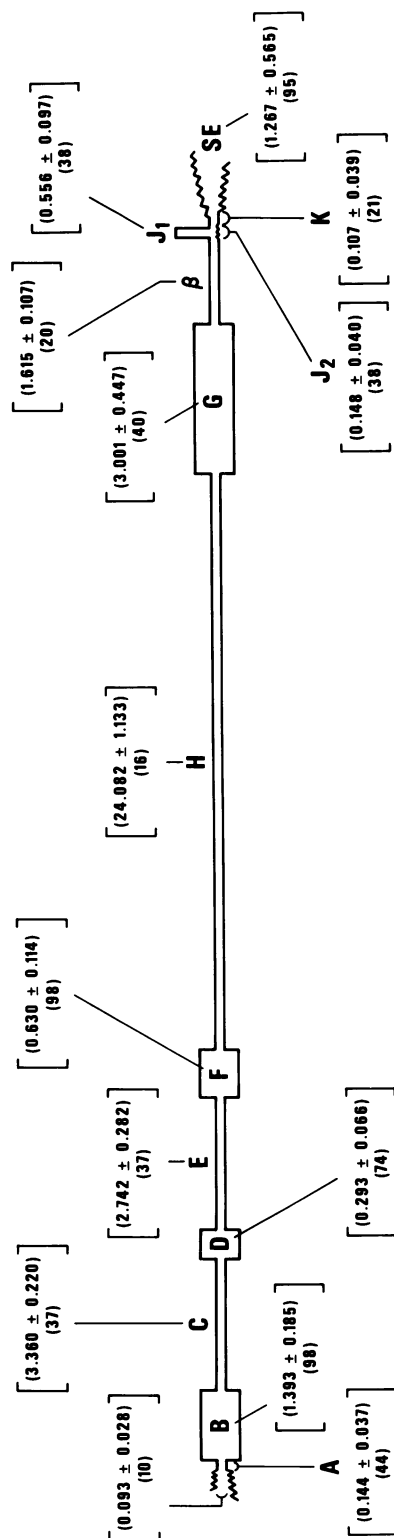


FIG. 5. Schematic sketch of the D108:Mu heteroduplexes. Average sizes of all the homologous and nonhomologous regions seen in the electron micrographs were computed from length measurements on a number of heteroduplex molecules using ϕ X174 ds and ϕ X174 ss as internal length standards. The average length (in kb) of each region and the number of molecules measured are shown in parentheses. The boxed letters B, D, F, and G indicate four of the five nonhomologous regions. The fifth nonhomology J, which is an insertion, has two unequal arms which are marked as J1 and J2. The squiggly lines at either end of the drawing indicate the bacterial DNA known to be covalently linked to Mu DNA. The sketch is not to scale.

Mu and D108, respectively, and comparing the gene products, Magazin et al. have shown that the 24,000-molecular-weight protein is seen only in Mu-infected minicells (21). They also found two proteins of molecular weight 56,000 and 23,000 that were present in D108-infected minicells only (21). The obvious conclusion from these results is that the corresponding *c* gene product is different in D108. The *A* gene of Mu, which is a gene to the right of the *c* gene on the Mu map, codes for a 70,000-molecular-weight protein (21). D108 also codes for a 70,000-molecular-weight protein (21). If the 70,000-molecular-weight protein is the product of the equivalent *A* gene in both D108 and Mu, the nonhomology B may span only the *c* gene and the intercistronic region, i.e., the region between the *c* and *A* genes. Recently, it has been shown that there might be another gene, called *ner*, located between the *c* and the *A* genes (10). Thus, it is possible that the nonhomology B spans the region containing the *c* and the *ner* genes. The presence of nonhomology B corroborates our earlier genetic studies establishing the heteroimmunity of D108 to Mu (13).

At present, the nonhomologies marked D and F cannot be ascribed to any other functional differences between the two phages. Having already discussed the differences in the G-loops, we will compare the β regions of the two phages. According to our measurements, the β region of D108 (β -D108) is 2.291 (~2.3) kb in length. The corresponding region of Mu (β -Mu) has been reported to be 1.6 to 1.7 kb in length (3, 5). The size of the β region computed from the D108:Mu heteroduplexes (without including the 556-bp insertion) is $(1.615 + 0.148 + 0.114 = 1.877)$ kb (Fig. 5). This means that β -D108 is ~400 bp longer than the corresponding region in D108:Mu heteroduplexes. These results lead us to conclude that the insertion is in the D108 DNA and not in Mu. Earlier studies of Bukhari and Taylor indicate that Mu, initiating packaging of its DNA at the left end (*c* end) of the molecule, matures its DNA by the headful (3). Our length measurements on D108 and Mu DNAs suggest that they are equal in size (Table 1). Therefore, if D108 matures its DNA by the headful also, the average length of the bacterial DNA associated with the D108 DNA at the SE should be shorter by $(2.3 - 1.9) = 0.4$ kb than the average length of bacterial DNA in Mu and Mu:D108 heteroduplexes. This, indeed, was found to be the case. The average length of the ss tails in D108 and D108:Mu heteroduplexes was 0.966 and 1.267 kb, respectively.

A closer examination of some of the D108 and D108:Mu heteroduplex molecules revealed that

there might be short stretches of unpaired sequences at the left end of the molecules (see Fig. 4b to the left of A). Occasionally, a small knob-like structure is seen instead of an SE. The average length of such tails measured from D108:Mu heteroduplexes is (98 ± 28) bp. Bukhari et al. have shown that the left end of Mu carries some bacterial DNA sequences 50 to 100 bp in length (1, 2). In this regard, D108 DNA would seem similar to Mu DNA.

The discovery of D108 and its close relationship to Mu suggest that, like the lambdoid group, there is a family of mutator phages. The availability of D108, which is heteroimmune to Mu and has a broader host range than that of Mu, should prove useful in numerous genetic studies.

ACKNOWLEDGMENTS

This work was supported by a research grant from the National Science Foundation (PCM-77-26477). G.S.G. received support from Public Health Service training grant T32 GM 07090-02 from the National Institute of General Medical Sciences and American Cancer Society fellowship PF-1395. R.A.H. received support from Public Health Service training grant T32 AI 07041-02 from the National Institute of Allergy and Infectious Diseases.

We would like to thank the art and photography section of The Upjohn Co. for generous help with the art work.

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