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Ar^+ Plasma-Induced Damage to DNA in Bacteriophage λ: Implications for the Arrangement of DNA in the Phage Head

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Bacteriophage λ was bombarded with low-energy Ar^+ ions with the goal of determining whether particular regions of the DNA genome are found preferentially in the outer portion of the packaged DNA mass. The strategy was to fragment the DNA selectively near the surface of the virus by exposing intact phage to Ar^+ ions energetic enough to break covalent chemical bonds in DNA but not energetic enough to penetrate deeply beneath the viral capsid shell. Broken DNA was then isolated, and its genomic origin was identified by Southern hybridization to mapped restriction fragments of λ DNA. Analysis of such Southern blots revealed that all regions of the λ genome were represented among the small DNA fragments generated during all times of Ar^+ bombardment examined. Depending on the duration of exposure, however, particular regions of the genome were found to be enriched in the small-fragment population. After short periods of exposure, sequences from the leftmost 10% and from the right half of the standard genetic map were enriched in the broken-DNA fraction. Among sequences in the right half of the genome, the enrichment was progressively more pronounced beginning in the middle of the genetic map and proceeding toward the right end. In phage bombarded for longer periods of time, rightward sequences were preferentially depleted in the small-fragment population. In contrast, when Ar^+ bombardment was carried out with free λ DNA rather than intact phage, small DNA fragments arose uniformly from all regions of the genome at all times of exposure examined. The results indicate that in the intact phage, DNA sequences from the right half and from the very leftmost regions of the genome have a tendency to lie closer to the capsid than does the remainder of the genome. Since DNA is packaged into the prohead beginning at the left end, our results suggest that packaging occurs in such a way that newly entering DNA tends to be disposed externally to that packaged at earlier times.

DNA is found in a highly condensed state inside the capsids of double-stranded DNA bacteriophages such as T4, λ, P22, and their relatives. The DNA concentration, for instance, has been calculated to be approximately 0.5 g/ml for most double-stranded DNA phages (6), and the DNA strands are packed very closely together, the spacing being 2.6 to 2.7 nm, as determined by small-angle X-ray diffraction (7, 8). This is comparable to the value of 2.8 nm (12, 16) found for the interstrand spacing in crystalline B-form DNA. Further, DNA is virtually the only component present inside the capsid of phage λ; proteins make up the capsid shell, but they are not found in significant quantities inside the capsid cavity. During the viral replication cycle, free DNA is inserted into an empty (i.e., DNA-free) protein shell called the procapsid. A single DNA molecule enters the procapsid at a unique site called the portal vertex, and the genome is then packaged linearly in a defined direction (1, 2, 20, 21).

Cryoelectron microscopic images of T4 and λ (15) show that in the intact phage, DNA is condensed into small domains where the DNA strands are aligned in parallel. The domains themselves, however, are oriented differently with respect to one another in individual virions, suggesting that one is not seeing different views of an invariant structure, but rather individual virions differ in the location of DNA-aligned domains. The cryoelectron microscopic images, therefore, demonstrate some local strand alignment in the packaged DNA, but they do not address the issue of long-range order.

Conflicting results have been obtained from studies that have directly tested for long-range order in packaged phage DNA. Ion etching studies of T4 and λ, for example, have indicated that the last DNA to enter the procapsid lies closer to the surface of the virion than does the DNA packaged earlier (3, 5). When intact phage is physically eroded in an Ar^+ plasma, the last DNA packaged is found to be lost more readily than is the remainder of the DNA. In contrast, cross-linking studies have suggested a more random distribution of DNA in phage λ. Widom and Baldwin (26) have demonstrated that any region of the λ genome can lie close enough to the capsid shell to be cross-linked to it by irradiation with UV light. Similarly, Haas et al. (13) have demonstrated that in the intact phage, any two regions of λ DNA can lie close enough to one another to be cross-linked by bis-psoralen.

The experiments described here were undertaken to clarify the issue of long-range order by use of a novel application of ion etching methodology. Intact λ phage was exposed to a low-energy (~1-keV) Ar^+ plasma whose ions are energetic enough to break covalent chemical bonds in DNA but not energetic enough to penetrate deeply beneath the capsid shell or to desorb (sputter) material from the virus surface. Under such conditions, DNA from the outermost regions of the DNA mass is expected to be damaged preferentially. We therefore isolated the first-damaged DNA and determined its location in the phage genome by Southern hybridization to mapped restriction fragments of whole λ DNA. Specificity in the genomic location of the first-damaged DNA was expected to be observed only if there exists some degree of long-range ordering in packaged λ DNA.

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MATERIALS AND METHODS

Phage growth and purification. Lambda phage was prepared from the temperature-inducible *Escherichia coli* lambda lysogen *c*I857 GM119 (Sigma Chemical Co.), using a procedure similar to that described by Maniatis et al. (19). In a 2-liter flask, 300 ml of LB broth was inoculated with an overnight culture of lambda *c*I857 GM119 and incubated at 34°C until the culture reached an optical density at 630 nm of 0.9. Phage growth was induced by raising the temperature to 45°C for 30 min. Thereafter, the temperature was lowered to 37°C and growth was continued for 3 h. Cells were then pelleted, resuspended in 10 ml of SM buffer (10 mM $MgSO_4$, 10 mM Tris [pH 7.5]), and lysed by addition of 0.5 ml of $CHCl_3$ and 2 μg of DNase per ml. This crude lysate was clarified by centrifugation for 5 min at 12,000 × *g* in a Sorvall SS-34 rotor, and phage were purified from the lysate by two steps of CsCl density gradient ultracentrifugation (19). The band of purified phage was removed from the gradient, dialyzed extensively against SM buffer to remove CsCl, titered by plaquing on a permissive host (*E. coli* C600), and diluted to 10^{12} PFU/ml with SM buffer for use in the experiments described below. Immediately prior to use, phage suspensions were made 0.2 mg/ml in bovine serum albumin to facilitate spreading of virions onto plastic coverslips. ^{32}P-labeled λ phage was prepared as described above except that prior to phage induction, 5 mCi of $H_3{}^{32}PO_4$ was added to 100 ml of culture.

Ar^+ bombardment. All phage and λ DNA specimens were subjected to Ar^+ bombardment after freeze-drying onto plastic coverslips (2.2 by 2.2 cm; Fisher Scientific catalog no. 12-547). For Ar^+ exposure of intact phage, each coverslip was spread evenly with 10 μl of SM buffer containing 10^{10} phage (enough to cover ~13% of the total coverslip surface) plus 0.2 mg of bovine serum albumin per ml and incubated for 10 min in a moist chamber to allow phage to adhere. Coverslips were then washed briefly (twice) in 10 mM ammonium acetate–0.1 mM magnesium acetate, frozen rapidly in liquid nitrogen, and dried in vacuo. Promptly after drying, coverslips were placed virus side up in half of a 100-mm-diameter plastic petri dish and exposed to an Ar^+ plasma as described below. DNA specimens (0.1 μg per coverslip) were prepared for Ar^+ bombardment in the same way except that coverslips were glow discharged prior to being spread with DNA and the washing steps were omitted. Ar^+ bombardment of freeze-dried specimens was carried out in a modified Polaron E5100 sputter coater whose properties have been described recently (5). Plasmas were produced in a cylindrical chamber 15 cm in diameter between two disk-shaped aluminum electrodes 14 cm in diameter and separated by 4 cm. Plasmas were developed at a current of 1 mA in 100% Ar at a pressure of 100 mtorr (13.33 kPa). The expected depth of ion penetration (2.2 to 4.4 nm) was derived from the results of a study (22) in which ions with energies comparable to those employed here were used to bombard palmitic acid multilayers containing a radioactive layer buried at different depths beneath the surface of the overall multilayer. The ion penetration depth (range) was considered to be the thickness of the nonradioactive palmitate cover required to protect the reference (radioactive) layer temporarily from damage by energetic ions.

DNA isolation and Southern hybridization. DNA was isolated from coverslips containing Ar^+-exposed phage or phage DNA by extraction overnight at 4°C in extraction buffer (6 M guanidine-HCl, 5 mM dithiothreitol, 10 mM Tris-HCl [pH 7.5]; 0.3 ml per coverslip). Control studies with radioactive phage or phage DNA demonstrated that this procedure resulted in solubilization of >92% phage DNA and >95% free DNA. Further analyses were performed with DNA pooled from three identically treated coverslips. DNA was recovered from extraction buffer with GeneClean (Bio 101, La Jolla, Calif.), subjected to electrophoresis overnight on a 0.85% agarose gel (20 by 25 by 0.5 cm) in TPE buffer (19) (2 V/cm) with molecular weight markers derived from *Hin*dIII-digested λ DNA, and visualized by staining the gel with 0.5 μg of ethidium bromide per ml. DNA fragments 1 kb or smaller were excised from each lane of the gel and prepared for use as probes in Southern hybridization experiments. DNA was isolated from gel slices with GeneClean and determined quantitatively with DNA Dipsticks (Invitrogen, San Diego, Calif.). Aliquots of 10 ng were copied into radioactive form, using the random priming method (10) with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham, Arlington Heights, Ill.) as a source of radioactive label. Probe specific activities were in the range of 5 × 10^5 to 1 × 10^6 cpm/ng for experimental samples from Ar^+-bombarded materials and 5 × 10^6 to 8 × 10^6 cpm/ng for unexposed whole λ DNA.

Southern hybridizations were carried out with DNA fragments produced by digestion of λ DNA with each of eight restriction endonucleases, *Acc*I, *Bcl*I, *Bgl*I, *Eco*RI, *Hin*dIII, *Mlu*I, *Nde*I, and *Nru*I. Two 0.3-μg aliquots of λ DNA were digested to completion with each of the eight enzymes, and the digests were subjected to electrophoresis on 0.85% agarose gels, as described above, in two identical blocks of eight lanes each. After electrophoresis, DNA was vacuum transferred from agarose gels onto supported nitrocellulose membranes (BAS-NC; Schleicher & Schuell, Keene, N.H.) for 4 to 6 h at a pressure of 30 cm of H_2O in an LKB Vacugene apparatus. Vacuum rather than capillary transfer was found to be essential to achieve the degree of uniformity of DNA transfer required for the experiments described below. When transfer was complete, the DNA was fixed to the membranes by baking at 80°C in vacuo for 1 h. The membranes were then cut in half to produce sets of two identical blots. The two blots were hybridized with probes prepared, respectively, from Ar^+-bombarded specimens or from whole λ DNA as described above. All hybridizations and washings were performed in screw-cap glass tubes (38 by 200 mm; Bellco Glass Co., Vineland, N.J.) in a rotating incubator set at 3 rpm. Hybridizations were carried out at 68°C overnight in 10 ml of hybridization buffer (19) (5× SSPE [5× SSPE is 0.9 M NaCl, 50 mM NaH_2PO_4, and 5 mM EDTA, pH 7.4], 5× Denhardt's solution, 0.5% sodium dodecyl sulfate [SDS], 150 μg of salmon sperm DNA per ml, 10% dextran sulfate). Thereafter, blots were washed twice at high stringency (30 min at 68°C in 0.1× SSPE–0.1% SDS), wrapped in Saran Wrap, and subjected to autoradiography with Kodak XRP film, using Cronex Hi-Plus intensifying screens.

Data analysis. Autoradiograms were digitized by scanning in an LKB UltroScan XL laser densitometer, and the resulting curves from experimental and control lanes (for each restriction enzyme digest used) were scaled to one another by a best-fit approach. Individual bands were then integrated by using a first-order Gaussian curve fit (with the LKB Gelscan program, version 1.2), and for each restriction fragment, the integrated area in the experimental band was expressed as a fraction of that in the control. At 1-kb intervals along the genome, an average experimental/control ratio was then computed from the relevant restriction fragments in as many as possible of the eight restriction digests;

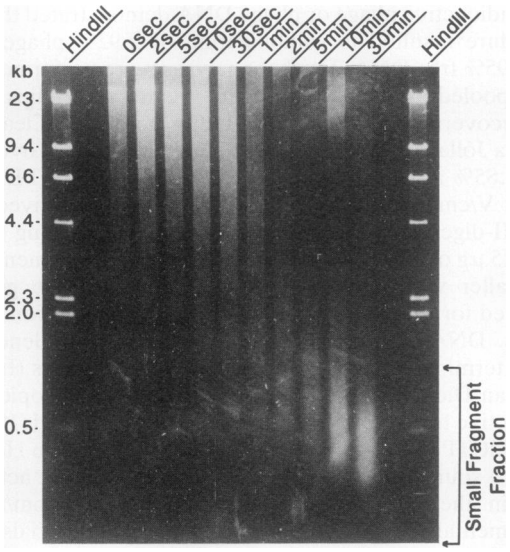

FIG. 1. Agarose gel electrophoresis of DNA isolated from Ar^+-bombarded phage λ. Intact phage was exposed to an Ar^+ plasma for various times as indicated above the lanes. DNA was then isolated from the phage and subjected to electrophoresis on a 0.85% agarose gel beside molecular weight markers derived from *Hin*dIII-digested λ DNA. The region of the gel indicated by the arrows, corresponding to the small-fragment fraction (1 kb and smaller), was excised from each lane and used as a probe in Southern hybridization experiments.

this average ratio was plotted as a function of position along the λ genome.

RESULTS

The experimental strategy used in this study depends critically on the properties of the Ar^+ plasma. As indicated above, the appropriate plasma must contain ions able to break covalent chemical bonds in DNA but not able to penetrate deeply beneath the virion surface or desorb DNA fragments. In practice, a plasma developed at a current of 1 mA was found to have the desired properties. Newcomb et al. (22) showed that ions in such a plasma penetrate biological materials to maximum depths of 2.2 to 4.4 nm or approximately one to two layers of DNA (cf. λ phage head diameter of ~65 nm [14, 15]). When λ phage containing ^{32}P-labeled DNA was exposed to a 1 mA Ar^+ plasma under the conditions used in this study, no statistically significant loss of ^{32}P label was observed after 30 min of bombardment (data not shown). Similarly, no evidence of surface erosion was detected when λ phage was bombarded for 30 min, shadowed with Pt-C, and examined in the electron microscope (data not shown).

Figure 1 shows the results obtained when DNA was isolated from Ar^+-bombarded phage and subjected to electrophoresis on 0.85% agarose gels. At all times of Ar^+ exposure tested, DNA was found to migrate in a broad band or smear of fragments that spanned a wide range of molecular lengths, e.g., 48 to <3 kb in phage bombarded for 10 s. The midpoint of the smear, however, shifted to a progressively lower molecular weight with longer durations of exposure, suggesting that smaller DNA fragments were being produced as phage absorbed more hits from energetic Ar^+ ions. The smallest DNA fragments migrated in a band that was centered at ~500 bp and spanned the range from

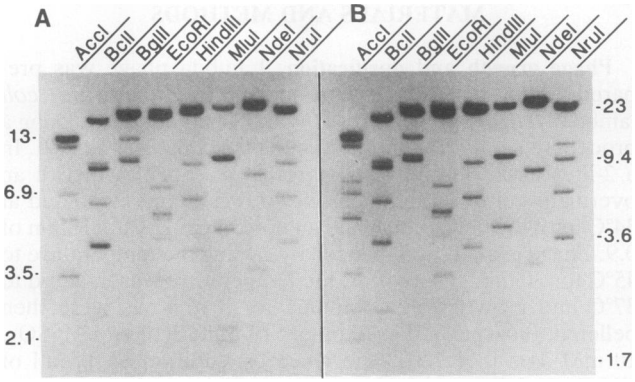

FIG. 2. Southern hybridization analysis of small DNA fragments produced during exposure of phage λ to an Ar^+ plasma. Blots containing mapped restriction fragments of λ DNA were probed with ^{32}P-labeled whole λ DNA (A) or ^{32}P-labeled small DNA fragments (1 kb and smaller) produced by subjecting lyophilized phage λ to an Ar^+ plasma for 30 s (B). Numbers at the left and right indicate molecular sizes of selected bands in kilobases.

~90 to ~1,000 bp. Thirty seconds was the shortest Ar^+ exposure time that produced DNA fragments migrating in this small-fragment fraction. After 30 min of bombardment, however, virtually all DNA was found in this region of the gel.

For purposes of the studies described below, damaged DNA is defined operationally as that migrating in the small-fragment fraction. This material was isolated from Ar^+-bombarded phage and radioactively labeled (10), and its genomic origin was determined by using it to probe a Southern blot containing mapped restriction fragments of λ DNA. As a control, free λ DNA was bombarded with Ar^+ under the same conditions used for phage, and the resulting small-fragment DNA was subjected to the same analysis (i.e., as a probe in a Southern hybridization experiment).

Figure 2B shows the autoradiograph obtained when a blot was probed with small-fragment DNA isolated from phage that had been bombarded with Ar^+ for 30 s. A sister blot probed with whole λ DNA is shown in Fig. 2A. The genomic locations of major restriction fragments are indicated in Fig. 3. By comparing Fig. 2A and B, it can be seen at a qualitative level that all restriction fragments labeled by the whole λ

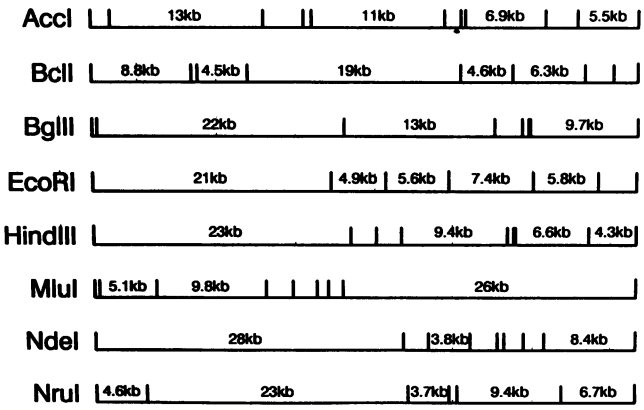

FIG. 3. λ DNA restriction map for the eight restriction enzymes shown in Fig. 2. Numbers indicate the approximate molecular sizes of the larger bands.

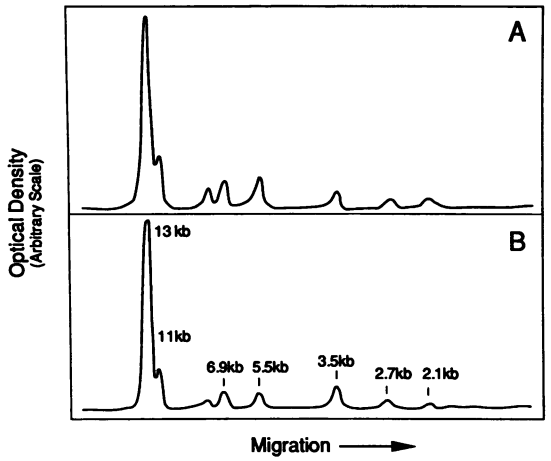

FIG. 4. Laser scans of the *Acc*I lanes shown in Fig. 2. ^{23}P-labeled DNA probes were derived from Ar^+-bombarded phage (A) or whole λ DNA (B). Numbers indicate the approximate molecular sizes of the major restriction fragments.

probe were also labeled by the probe prepared from ion-bombarded phage. Quantitatively, however, significant differences were found to exist in the intensity with which corresponding bands were labeled. For example, the *Acc*I 5.5-kb and the *Hin*dIII 4.3-kb fragments were more heavily labeled in the experimental blot than in the control blot, while the opposite pattern was observed in the case of the *Mlu*I 2.2- and 2.0-kb fragments. Such quantitative differences were seen more clearly when autoradiographs were digitized by scanning in a laser densitometer and corresponding traces were scaled to each other. An example of such an analysis is shown in Fig. 4 for the *Acc*I lanes in Fig. 2. Here it can be seen that the 5.5- and 6.9-kb bands were labeled more heavily by the experimental probe than by the control probe, while the extents of labeling of the 3.5- and 13-kb bands were more nearly equal.

To integrate information from all eight restriction digests, the data were processed as follows: (i) all autoradiographs were digitized, and corresponding traces were scaled; (ii) for each labeled band, the integrated intensity (peak area) in the experimental blot was divided by the corresponding value in the control; and (iii) the latter ratio was averaged among the eight restriction digests at 1-kb intervals along the λ genome, and the value was plotted as a function of genome position as shown in Fig. 5. Such plots were produced for all Ar^+ exposure times at which recovery of DNA from the small-fragment fraction was above background (i.e., ≥30 s).

The plots were found to be of two different types, depending on whether phage were exposed to Ar^+ for short (30 s to 2 min; Fig. 5A) or long (5 to 30 min; Fig. 5B) times. In phage bombarded for short times, the small-fragment fraction was systematically enriched in sequences derived from the right half of the standard genetic map and in sequences from the leftmost ~5 kb of the genome. Among the rightward sequences, enrichment was progressively greater beginning at approximately the middle of the genome and proceeding to the right end. The highest experimental/control ratios were ~1.75 (Fig. 5A). In contrast, when phage were bombarded with Ar^+ for 5 min or longer, sequences from the left half of the genome were enriched in the small-fragment population while rightward ones were depleted. Enrichment was reasonably uniform over the leftmost half of the genome.

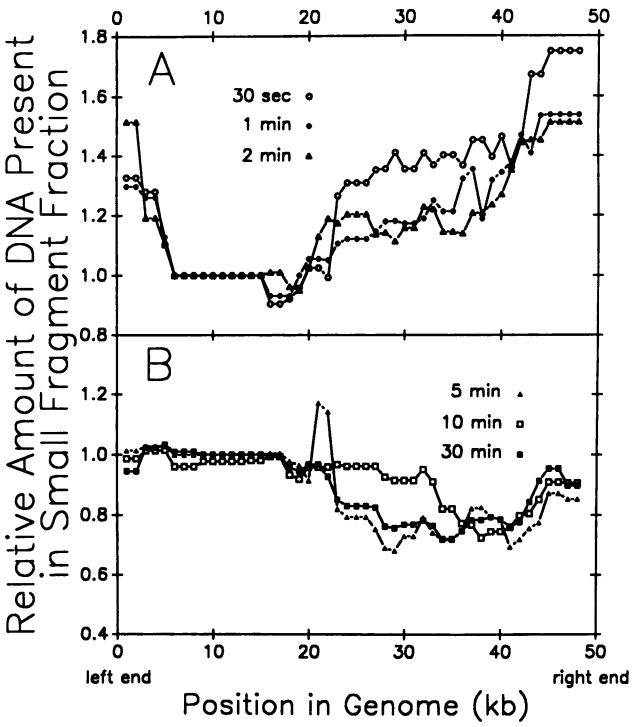

FIG. 5. Genomic origin of the small DNA fragments produced during Ar^+ bombardment of phage λ. The relative composition of the small DNA fragment fraction was determined by laser densitometry of autoradiograms from Southern blots such as those shown in Fig. 1. The integrated intensity (peak area) of each labeled restriction fragment was expressed as a ratio to the control and plotted with respect to genomic position. Points on the plots represent averages of the experimentally determined ratio for the restriction fragments that overlapped a given 1-kb interval of the genome. (A) Relative composition of the small DNA fragments generated by exposing phage to an Ar^+ plasma for 30 s, 1 min, and 2 min. (B) Relative composition of the small DNA fragments generated by exposing phage to an Ar^+ plasma for 5, 10, and 30 min.

Quantitatively, the degree of enrichment in leftward fragments was slightly less than that observed for rightward species at short Ar^+ exposure times.

A quite different pattern of labeling was observed when free λ DNA rather than intact phage was subjected to Ar^+ bombardment. In this case, the small-fragment fraction was found to contain a nearly random subset of the λ genome at all Ar^+ exposure times tested. Figure 6 shows representative results obtained when DNA was bombarded for short (30 s) and long (30 min) times. In studies with free DNA, the experimental/control ratio was found to be within the range of 0.91 to 1.06 for all fragments tested (i.e., in eight restriction endonuclease digests). The comparable range for studies with intact phage was 0.68 to 1.75.

DISCUSSION

The purpose of this study was to determine whether there exists long-range ordering in the arrangement of DNA in the head of bacteriophage λ and, if so, to consider the way(s) in which it may arise. Our experimental approach was to use energetic Ar^+ ions, as found in ion plasmas, to introduce breaks selectively into the outermost strands of the packaged DNA mass. The first DNA damaged was then isolated,

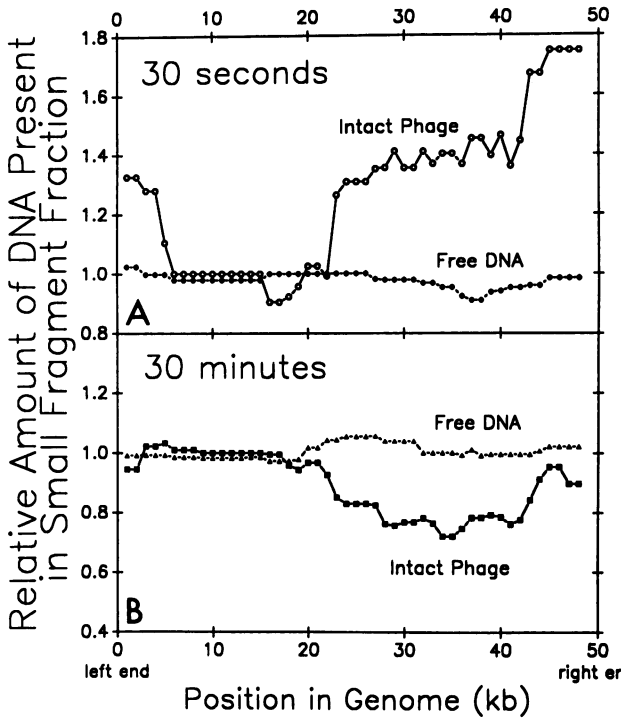

FIG. 6. Genomic origin of the small DNA fragments produced during Ar^+ bombardment of free genomic λ DNA. Lanes of autoradiograms were scanned by densitometry and quantitated as described in Materials and Methods. (A) Relative composition of the small DNA fragment population produced by Ar^+ bombardment of free λ DNA for 30 s compared with that produced by similar bombardment of intact phage for the same time; (B) similar plots for free DNA and phage exposed to the Ar^+ plasma for 30 min.

and its origin along the λ genome was identified by Southern hybridization experiments. The results demonstrate that DNA sequences from all regions of the genome occur in the broken-DNA fraction. This was the case with the first DNA damaged by the gentlest Ar^+ bombardment able to cause breaks in DNA. Therefore, we conclude that all regions of the λ genome can reside in the external regions of the packaged DNA mass within the range (one to two layers of DNA) of the Ar^+ ions used in this study. Quantitative analysis of the Southern hybridization experiments, however, revealed that all λ DNA sequences were not equally represented in the first-damaged population. Sequences from the right half of the genome and from the leftmost 5 kb were significantly enriched. This finding suggested that they are more likely than the remainder of the genome to be located in the external portions of the packaged DNA. Since no comparable enrichment was observed when free λ DNA rather than intact phage was exposed to Ar^+, we conclude that the enrichment observed is due to the way in which DNA is arranged in the phage head and not to an inherently greater sensitivity of rightward sequences to breakage by energetic ions.

When phage were exposed to the ion plasma for long (i.e., ≥5 min) rather than short periods of time, the composition of the damaged-DNA fraction changed significantly; rightward sequences were found to be depleted rather than enriched. We assume that depletion of rightward sequences resulted from the accumulation of Ar^+-induced damage to the outermost DNA in the phage head. Such accumulated damage would alter the most external DNA too extensively for it to be copied accurately into a ^{32}P-labeled DNA probe. Thus, the less-damaged leftward sequences would be expected to predominate in Southern hybridization experiments as we have observed. This interpretation of our results is consistent with the view that rightward regions of the genome are enriched in externally disposed DNA while leftward ones are more prevalent toward the center of the phage head.

Our results suggest that both ends of the genome tend to be found at the periphery of the DNA mass. This result agrees with experiments involving tailless mutants of P2 (18) and P4 (17, 27). When DNA is isolated from these mutants, the genome is recovered as a topologically knotted structure with the cohesive ends annealed. This may indicate (2) that the two ends of the genome are positioned near one another and at the portal vertex. Our results suggest this may be the case with phage λ as well. The latter conclusion was not anticipated. In phage T4, in which the first end of the DNA packaged is also the first end ejected (4), it is expected that both termini must be positioned at the surface of the DNA mass (1, 2, 4). However, in λ, the first end of the DNA packaged is the last ejected (9, 11, 25). It might have been anticipated that in λ, the left terminus of the genome would be found at the center of the virus. Instead, both termini appear to be located at the periphery of the packaged DNA mass. This finding provides support for the idea that there is a unitary packaging structure utilized by all double-stranded DNA bacteriophages (1, 2, 5).

The results reported here are in agreement with those obtained previously in Ar^+ plasma etching studies of phages T4 and λ. For example, in the case of T4, the last DNA packaged into the prohead (which corresponds to rightward DNA in λ [9, 11, 24]) was found to be lost more rapidly than the remainder when the virus surface was physically eroded by energetic ions (3). Similarly, rightward DNA was selectively lost when the overall size of phage λ was reduced by etching in an Ar^+ plasma (5). The present studies have added the idea of a gradient of proximity to the capsid shell among rightward sequences and evidence that the very leftmost portion of the λ genome is enriched in more-external DNA.

The present studies also complement previous analyses in the way in which ion plasmas were used. In previous studies (3, 5), the virus surface was eroded entirely (sputtered) by the action of energetic Ar^+ ions, and analyses were performed with the DNA remaining. The identity of external DNA was inferred as the difference between the whole and what remained after etching. In contrast, the experimental approach used in the present study does not involve physical loss of phage DNA. Rather, capsid-proximal DNA was able to be physically isolated and identified directly.

The tendency of last-packaged DNA to lie near the capsid shell as described here (and in previous studies [3, 5]) most probably has its origin in the way DNA enters the phage prohead. Once the head is filled, DNA strands are unlikely to rearrange significantly. Since λ DNA is packaged beginning at the left end (9, 11, 24), the observed right end external–left end internal polarity would be explained if DNA filled the prohead in such a way that newly entering DNA tended to be disposed externally to that already present. The last DNA to enter (i.e., the right end) would therefore be found on the external surface of the overall condensate. In its pure form, such an external-preferred packaging scheme must, however, constitute a simplification of the real situation. As shown by Widom and Baldwin (26) and by the present study, all regions of the λ genome can occur in the outermost portions of the mature DNA mass. As this latter observation

is clearly not compatible with a straightforward internal-to-external packaging scheme, we conclude that some qualification to the simple form of the mechanism must apply. Two possibilities suggest themselves. (i) One is to suppose that the DNA in general enters the prohead externally to DNA packaged earlier, but it is able to become rearranged to some extent thereafter. The extent of rearrangement would presumably decrease progressively as the prohead is filled with DNA. The result would be a population of mature phage with considerable individual variation in the way the DNA is arranged. In all, however, there would be a tendency for the last-packaged DNA to reside nearer to the capsid shell. (ii) Alternatively, DNA may be arranged in the phage head in such a way that it makes periodic excursions from the interior to the exterior of the overall packaged mass. This concept is embodied in the spiral-fold and related models of DNA packing (3, 23). Since the λ head is ~65 nm in diameter, the required excursions could be accomplished with DNA lengths of <120 bp.

The existing electron micrographs of mature T4 and λ preserved in the frozen-hydrated state (15) are probably compatible with both DNA packaging schemes described above. Further information about DNA arrangement might, however, be obtained if one could produce electron microscopic images of proheads preserved in the frozen-hydrated state while in the process of being filled with DNA. Such micrographs should at least clarify the issue of whether DNA fills the capsid cavity isotropically as packaging proceeds or whether it is condensed in a specialized area such as the portal vertex or the inner surface of the capsid wall.

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