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Optimal Lengths for DNAs Encapsidated by Epstein-Barr Virus

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We measured the efficiency of DNA packaging by Epstein-Barr virus (EBV) as a function of the length of the DNA being packaged. Plasmids that contain *oriP* (the origin of latent EBV DNA replication), *oriLyt* (the origin of lytic EBV DNA replication), the viral terminal repeats (necessary for cleavage and packaging by EBV), and various lengths of bacteriophage lambda DNA were introduced into EBV-positive cells. Upon induction of the resident EBV's lytic phase, introduced plasmids replicated as concatemers and were packaged. Plasmid-derived concatemers of DNA with certain lengths were found to predominate in isolated virion particles. We measured the distribution of lengths of plasmid concatemers found within cells supporting the lytic phase of the viral life cycle and found that this distribution differed from the distribution of lengths of concatemers found in mature virion particles. This finding indicates that the DNA packaged into mature virions represents a selected subset of those present in the cell during packaging. These observations together indicate that the length of DNA affects the efficiency with which that DNA is packaged by EBV. Finally, we measured the length of the packaged B95-8 viral DNA and found it to be approximately 165 kbp, or 10 kbp shorter than the originally predicted size for B95-8 based on its sequence. Together with the results of other studies, these findings indicate that the packaging of DNAs by EBV is dependent on two imprecisely recognized elements: the viral terminal repeats and the length of the DNA being packaged by the virus.

The life cycle of Epstein-Barr virus (EBV) consists of a latent phase and a lytic phase. Its latent phase is established upon infection of B lymphocytes, during which a small subset of viral genes is expressed (8, 10). In latency, the viral DNA is maintained as a plasmid, and DNA synthesis is initiated from its origin of plasmid replication, *oriP* (15). Replication is semiconservative and synchronous with cellular DNA synthesis (1, 14). No infectious virus is produced during this phase. Occasionally the relationship between the host cell and the resident virus changes such that the lytic phase of the viral life cycle is induced. During this phase, the viral genome is amplified via DNA replication which initiates from EBV's origin of lytic replication, *oriLyt*, a *cis*-acting element distinct from *oriP* (6). Lytic replication from this site produces linear concatemers of the viral genome which may be synthesized via a rolling-circle mechanism (6). The linear concatemers are cleaved site specifically and packaged into capsids (7). These capsids mature and are released as viral particles by unknown mechanisms. We have measured the efficiency of DNA packaging by EBV as a function of the length of the DNA being packaged in order to analyze the regulation of this facet of EBV maturation.

Our experimental strategy used subgenomic plasmids that are replicated as concatemers and subsequently packaged by the virus. These plasmids contain *oriP* as well as a selectable marker so that they can be maintained in EBV-positive B-cell lines; they contain *oriLyt* so that they can be amplified upon induction of the lytic phase of the life cycle of the endogenous EBV; and they contain the terminal repeats (TR) of the linear viral genome, which are required for cleavage and packaging by EBV (7). Various lengths of bacteriophage lambda DNA were also inserted into the plasmids to provide a range of concatemeric lengths (Fig. 1). Cells were induced to support the lytic phase of EBV's life cycle, and concatemers of the plasmids were packaged by the virus. Viral particles were subsequently isolated, concentrated, and disrupted to release the DNA within them. Concatemeric plasmid DNAs were separated by length using pulsed-field gel electrophoresis (PFGE), transferred to nylon filters, and hybridized with specific radioactive probes. The signals generated by the packaged concatemers were detected by autoradiography and quantified by Phosphorimager analysis.

We have observed that (i) the efficiency of DNA packaging by EBV is dependent on the length of the DNA being packaged, such that a specific range of DNA lengths is packaged more efficiently than lengths that fall either above or below this range; (ii) the distribution of DNA concatemers within the cell during packaging differs from the distribution of concatemers packaged into mature virion particles; and (iii) the endogenous DNA packaged by the B95-8 strain of EBV is approximately 165 kbp in length, which is 10 kbp shorter than was previously predicted (3).

MATERIALS AND METHODS

Cells. B95-8 is a nonadherent lymphoblastoid cell line derived from a marmoset cell immortalized by EBV (11). Cells were propagated in RPMI with 10% bovine calf serum plus 200 μg of streptomycin per ml and 200 U of penicillin per ml.

Plasmids. p588 was constructed by inserting the TR (nucleotides 169423 to 644 of the B95-8 genome) of EBV into plasmid 562. p562 contains the origin of latent replication (*oriP*), the origin of lytic replication (*oriLyt*), and the selectable marker hygromycin B phosphotransferase (described in reference 6). To provide a wide range of plasmid lengths, lambda DNA sequences of various lengths were inserted into p588. Lambda sequences of 2.0, 2.3, and 6.3 kbp in length were each ligated individually into the *Hin*dIII site of p588 (Fig. 1), creating p588 (2.0 λ), p588 (2.3 λ), and p588 (6.3 λ). The approximate monomeric lengths of p588 and these lambda-containing derivatives are as follows: p588, 16.0 kbp; p588 (2.0 λ), 18.0 kbp; p588 (2.3 λ), 18.3 kbp; and p588 (6.3 λ), 22.3 kbp. p554 (28.8 kbp) was derived from p135, which contains the contiguous EBV sequences of the B95-8 strain that lies be-

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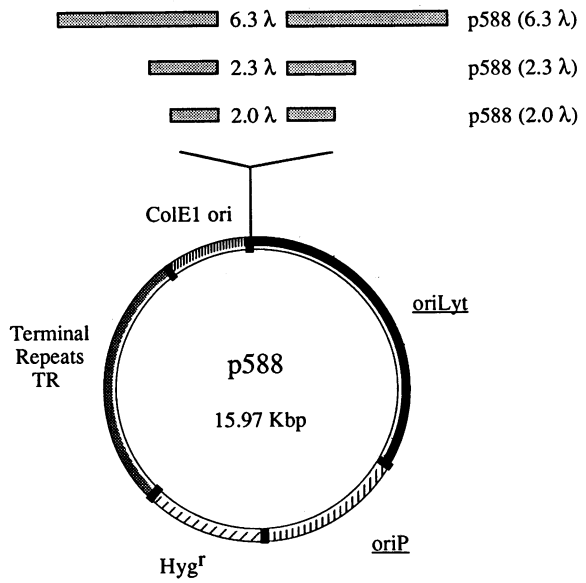

FIG. 1. Structure of p588. p588 for the generation of concatemers is based on p562 (6). p588 contains those *cis*-acting elements of EBV required for latent replication of the plasmid in EBV-positive cells (*oriP*) and for replication during the lytic phase of the viral life cycle (*oriLyt*), as well as the sequences necessary for cleavage and packaging (TR). Also included on the plasmid is a gene that provides hygromycin resistance (hygromycin B phosphotransferase; Hyg^r), as well as the ColE1 origin of replication (ColE1 ori). Three derivatives of p588 that contain lambda DNA fragments of 2.0, 2.3, and 6.3 kbp resulting from *Hin*dIII digestion of lambda DNA were constructed.

tween the *Eco*RI site at nucleotide 7315 and the *Sal*I site at nucleotide 56081 (7). p554 was derived through the addition of the TR of B95-8 into a unique *Nru*I site in p135 (7).

Electroporation and maintenance of plasmids in cells. Plasmids were introduced into the B95-8 cell line by using a University of Wisconsin electroporator and the following settings as described by Knutson and Yee (9): 1,500 V, three capacitor banks, *R* adjustment at 100%. These settings produced the following electric pulse characteristics; rise time = 700 ns; fall time = 35 ms; peak voltage = 740 V. Cells containing the plasmids were selected in RPMI with 10% calf serum, 200 μg of streptomycin per ml, 200 U of penicillin per ml, and 1 mg of hygromycin per ml at 37°C and 5% CO_2.

Induction of the lytic phase and isolation of viral particles. Cells containing p588 or one of its derivatives were grown to a density of 10^6 cells per ml under hygromycin selection and then induced to support the lytic phase of EBV by exposure to 3 mM Na butyrate and 20 ng of 12-*O*-tetradecanoylphorbol-13-acetate per ml for 5 days. Cell debris was removed by centrifugation of the culture supernatant at 2,000 rpm (600 × *g*) for 20 min and filtration through an 0.8-μm-pore-size filter (Nalgene) to remove remaining viable cells and cell debris. Viral particles were pelleted by centrifugation at 27,000 rpm (65,000 × *g*) of 240 ml of supernatant for 1 h at 4°C in an SW27 rotor. Pellets were resuspended in 12 ml of buffer (0.01 M Tris-HCl [pH 7.5], 0.15 M NaCl, 0.001 M EDTA [pH 8.0], 100 μg of bovine serum albumin per ml) overnight at 4°C. To remove unencapsidated DNA, DNase I (10 U/ml) (Boehringer Mannheim) and $MgCl_2$ (final concentration, 0.01 M) were added and the mixture was incubated at room temperature for 30 min. EDTA (0.02 M) was then added to inhibit DNase activity. To remove the virion envelope, 0.5% deoxycholate and 1% Triton X-100 were added. The particles were pelleted through a 25% sucrose cushion (25% sucrose, 0.001 M EDTA [pH 8.0], 0.15 M NaCl) by centrifugation at 24,000 rpm (52,000 × *g*) for 1 h at 4°C in an SW27 rotor and then resuspended in 250 μl of TE (0.01 M Tris-HCl [pH 7.5], 0.002 M EDTA [pH 8.0]) overnight at 4°C. The particles were concentrated to a volume of 100 μl (Centricon 30; Amicon) and added to 100 μl of liquid 2% high-grade agarose (Bio-Rad). The mixture was placed into an agarose plug mold and allowed to cool. The plug was stored in 0.025 M EDTA (pH 8.0) at 4°C.

Isolation and detection of concatemeric DNA packaged by the B95-8 strain of EBV. Agarose plugs containing virion particles or cells were treated to release the DNA by incubating in 5 ml of NDS buffer (0.01 M Tris-HCl [pH 7.5], 0.05 M EDTA [pH 8.0], 1% laurylsarcosine, 0.5 mg of proteinase K per ml) overnight at 50°C. The agarose plug was washed three times in 0.05 M EDTA (pH 8.0) for 15 min per wash and then incubated again in 5 ml of NDS buffer at 50°C overnight. The agarose plug was washed in 0.05 M EDTA (pH 8.0) once and soaked in EDTA overnight. The EDTA wash was changed, and the plug was stored at 4°C in 0.05 M EDTA (pH 8.0).

p554 concatemers present in B95-8 cells 3 days after induction of the lytic phase were analyzed for protection against endonuclease digestion. B95-8 cells containing p554 were isolated, pelleted, and placed in an agarose plug as described earlier. The plug was treated with 1% Nonidet P-40 (NP-40), 0.01 M EDTA (pH 8.0), and 0.01 Tris-HCl (pH 7.5) for 5 h at room temperature. The plug was washed three times in 1% NP-40–0.01 M Tris-HCl (pH 7.5) for 15 min each time. Approximately 50% of the plug was removed and treated with *Mlu*I at 1,000 U/ml (New England Biolabs) in the presence of 1% NP-40, 0.01 M Tris-HCl (pH 7.5), and 0.01 M $MgSO_4$ overnight at 37°C. *Mlu*I was inactivated at 65°C for 20 min. The plug was treated with NDS buffer overnight at 50°C and then washed in 0.025 M EDTA (pH 8.0). DNA concatemers from the plug treated with NP-40 and the plug treated with NP-40, *Mlu*I, and proteinase K were separated by PFGE on a 1% high-grade agarose gel (Bio-Rad). The gel was transferred to a nylon filter for Southern blot analysis (12) using pHyg (13) as a probe. Signals were detected by autoradiography.

Generation of molecular mass standards from p588. Molecular mass standards were generated by ligation of p588 that was linearized by digestion with *Hin*dIII (Boehringer Mannheim). Linear p588 (6 μg) was added to 1 μl of 10× T4 DNA ligase ligation buffer (U.S. Biochemicals), 2 μl of 40% polyethylene glycol 8000, and 4 μl of H_2O. This ligation stock (0.5 μl) was added to 1 U of T4 DNA ligase (U.S. Biochemicals), and incubated for 40 min at 15°C. The reaction mixtures were brought to a volume of 100 μl with TE, mixed with liquid 2% high-grade agarose (Bio-Rad), and poured into a plug mold. p588 molecular mass standards ranged from a 1-mer (16 kbp) to a 13-mer (208 kbp), and lengths were confirmed by comparison with λ DNA molecular mass standards (Clontech).

PFGE. DNA released from the virion particles contained in the agarose plug was separated by PFGE in a 1% high-grade agarose gel (Bio-Rad). The CHEF-DR II PFGE system (Bio-Rad) and a two-stage protocol with the following parameters was used: stage 1, switch time = 16 s and run time = 16 h; stage 2, switch time = 17 s and run time = 18 h. The voltage for both stages 1 and 2 was 140 V. The buffer temperature for both stages 1 and 2 was 10°C. After PFGE, the DNA was transferred to nylon filters.

Analysis of plasmid and B95-8 DNA packaged into virions. To detect concatemers of p588-based plasmids, the filters were hybridized with pHyg (13). To detect B95-8 DNA, the filters were stripped by washing in 0.4 M NaOH for 15 min at 37°C

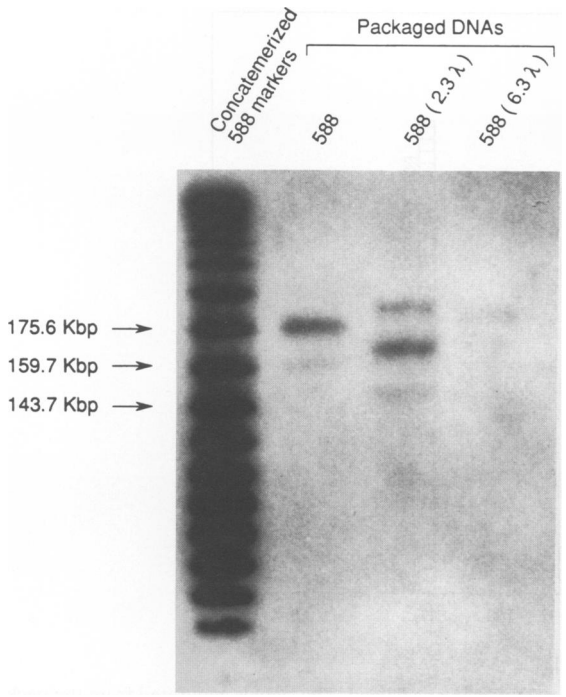

FIG. 2. Packaging of concatemeric DNAs by EBV. DNA packaged into virions from cells containing p588, p588 (2.3 λ), or p588 (6.3 λ) was fractionated by PFGE, transferred to nylon filters, hybridized with pHyg (13), and detected by autoradiography. The molecular mass standard is a series of p588 concatemers generated by ligation of p588 monomers in vitro, and their lengths are denoted in the figure. The first visible concatemer of the molecular mass standard is the dimer of p588. The approximate lengths of the p588-based packaged concatemers are calculated to be the following (from top to bottom of each column): p588 (11-mer), 176 kbp; p588 (10-mer), 160 kbp; p588 (2.3 λ) (10-mer), 183 kbp; p588 (2.3 λ) (9-mer), 165 kbp; p588 (2.3 λ) (8-mer), 146 kbp; and p588 (6.3 λ) (8-mer), 178 kbp (this signal is weak and is detected more efficiently in Fig. 5). It is important to note that the intensities of the signals generated by the packaged concatemers vary as a function of their length.

with shaking and then washed twice in 2× SSPE (20× SSPE is 3 M NaCl, 0.2 M NaH_2PO_4, and 0.02 M EDTA, adjusted to pH 7.4 with NaOH) for 15 min at room temperature with shaking. The filters were hybridized with linearized p925, which contains EBV sequences from positions 163477 to 3955 in pUC 19. Signals were detected and quantified with a Phosphorimager (Molecular Dynamics).

RESULTS

EBV-based plasmids are packaged as concatemers. p588 and its derivatives were transfected individually into the B95-8 cell line, and their encapsidated progeny were analyzed. Figure 2 represents analyses of the parental p588 (~16 kbp) and two of its derivatives, p588 (2.3 λ) and p588 (6.3 λ), containing inserts of 2.3 and 6.3 kbp of lambda DNA, respectively. Concatemers of these DNAs were cleaved and packaged by the helper virus into virion particles, and the lengths of the concatemers and the distribution of the lengths were found to vary among the p588-based plasmids. Similar analyses were performed with p588 (2.0 λ) (data not shown).

Only certain concatemeric lengths of DNA were packaged detectably by EBV. For p588, the 11-mer (~176 kbp) was present in virion particles more often than the 10-mer (~160 kbp), which was present more often than the 9-mer (~144 kbp) (Fig. 2). Virion particles encapsidating p588 (2.3 λ) contained the 9-mer (~165 kbp) more often than either the 10-mer (~183 kbp) or the 8-mer (~146 kbp) (Fig. 2). Finally, particles encapsidating p588 (6.3 λ) contained the octamer (~178 kbp) most often (Fig. 2). These measurements indicated that populations of DNAs with similar concatemeric lengths derived from different monomeric parental plasmids are encapsidated most frequently by EBV.

One concern in the design of these experiments was that insertion of lambda phage DNA might have an effect on the packaging efficiency of the resulting plasmid. As an internal control for packaging efficiency in each experiment, the Southern blots used to visualize the packaged DNAs of p588 and its derivatives were stripped and reprobed for B95-8 DNA. The B95-8 genome was present because it was encapsidated in the cells induced to support the viral lytic cycle and copurified with the particles containing p588-derived concatemers. The B95-8 DNA was separated from the plasmid DNA during PFGE and was visualized with a probe for B95-8 DNA which detected p588 and its derivatives inefficiently. By measuring the amount of B95-8 DNA present in each experiment relative to the packaged p588 DNA, the ratio of p588 DNA to B95-8 DNA packaged in each experiment was established. The ratio of packaged DNA of p588 or one of its lambda-containing derivatives to packaged B95-8 DNA was similar in all experiments (data not shown). These observations indicate that concatemers of the different plasmids were packaged with similar efficiencies, and therefore the lambda plasmid DNA does not affect detectably the efficiency of packaging by EBV.

The probe (p925) used to detect B95-8 DNA in these experiments recognizes approximately 13 kbp of B95-8 DNA and 6 kbp of the p588-based plasmids (see Fig. 5). The extent of the overlap of this probe with the detected DNAs and the equivalence of the signals detected in Fig. 5 for B95-8 and p588 indicate that these DNAs were packaged at a ratio of five to one. Given that the number of B95-8 genomes resident in uninduced cells is likely to be greater than the number of p588 molecules, the efficiency of packaging of the p588-derived plasmids after induction is high.

The efficiency of DNA packaging by EBV depends on the length of the DNA being packaged. The experiments described above were repeated for p588 and its derivatives. Figure 3 is a compilation of results representative of the four p588-based plasmids. The results indicate that EBV encapsidates p588-derived DNAs ranging from 73 to 183 kbp in length. Within the range of DNA lengths encapsidated by EBV, there exists a smaller range of lengths that are packaged more efficiently than those lengths that fall either above or below this range. In these experiments, 75% or more of the molecules derived from each plasmid that were isolated from mature virion particles were between 161 and 179 kbp in length (Fig. 3). Similar results were obtained with p554 (7), which is 28.8 kbp in length (data not shown). Specifically, approximately 97% of the signal generated by p554-based concatemers was detected at the hexamer length of 172 kbp, while roughly 3% of the signal was detected at the pentamer length of 144 kbp. We also detected the inefficient packaging of shorter concatemers of p554 DNA ranging from the monamer (28.8 kbp) to the tetramer (115 kbp). These concatemers each constituted less than 0.1% of the total signal generated by the p554-based concatemers packaged by the B95-8 strain of EBV.

The distribution of intracellular concatemeric DNA differs from that packaged into mature virions. The distribution of p588 concatemers detectable in cells in which the viral lytic

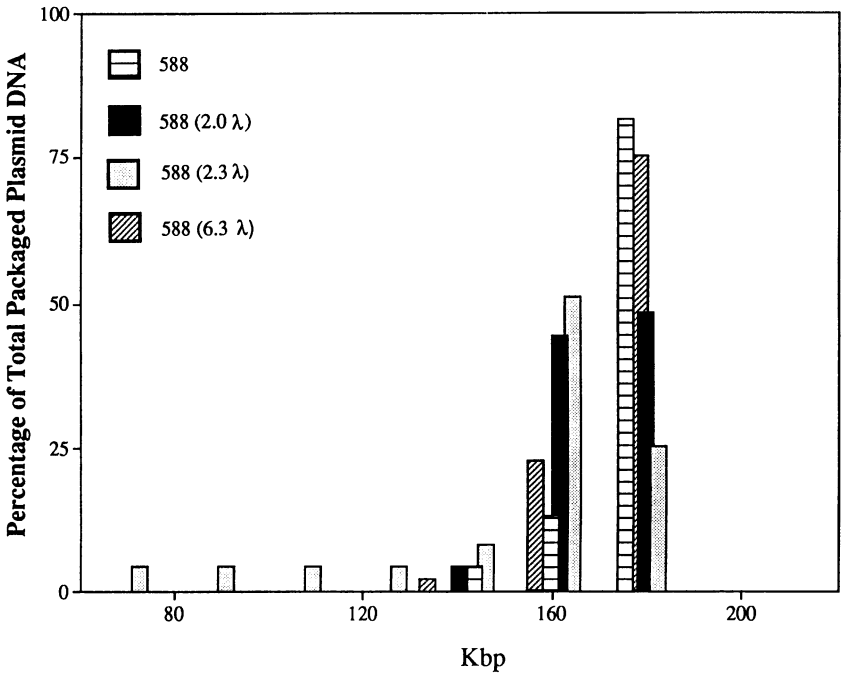

FIG. 3. The efficiency of DNA packaging by EBV depends on the length of the DNA being packaged. The signals generated from the packaged concatemers in these experiments were quantified relative to each other by Phosphorimager analysis (Molecular Dynamics). The concatemers derived from the four p588-based plasmids were packaged and analyzed independently. Shown are the percentage of each packaged concatemeric form derived from one plasmid relative to all the packaged concatemeric forms derived from the same plasmid and the length of the packaged concatemers measured. Each bar in the graph represents a unit-length multimer of the plasmid used in that particular experiment [e.g., the 10-, 9-, and 8-mers of p588 (2.0 λ) are represented by the black bars]. The graph indicates that the lengths of p588-based DNAs packaged detectably by the virus in these experiments range from 73 to 183 kbp, with a range of DNA lengths from 161 to 179 kbp packaged more efficiently by EBV relative to lengths that fall above or below this range.

cycle was induced was determined and compared with the distribution of concatemers detected in mature virion particles. B95-8 cells carrying p588 were induced to support the lytic phase and maintained for 3 days. At that time, greater than 90% of the cells were still viable as measured by their exclusion of a vital dye. The cells were pelleted, lysed, and analyzed for the presence of p588 concatemeric DNA. Shown in Fig. 4 is a comparison of concatemeric DNA found in B95-8 cells that have supported the lytic phase for 3 days with concatemeric DNA found in mature virion particles in supernatants from the same culture. These distributions differed. The p588 concatemers found in cells supporting the lytic phase of EBV's life cycle ranged from 128 to 176 kbp in length, and these concatemers appeared with approximately equal intensities. In addition, there appeared to be concatemers of much greater length (>213 kbp). No concatemers shorter than 128 kbp generated distinct, detectable signals within the cells. The distribution of p588 concatemers found within mature particles, however, shifted dramatically, showing a predominance of the 11-mer (176 kbp) concatemer, with the 10-mer (160 kbp) and the 9-mer (144 kbp) present in lesser amounts (Fig. 2, lane 588). No packaging of concatemers of p588 DNA larger than 176 kbp was observed. This experiment was repeated (data not shown), and the observations indicate that EBV encapsidates concatemeric lengths of p588 differing in distribution from that present in the cell during viral maturation.

Experiments were also performed to determine whether intracellular concatemers are resistant to digestion with an endonuclease prior to treatment with a protease. Such resistance would be consistent with the concatemers being protected by their association with capsids during the early stages of packaging. B95-8 cells transfected with p554 were induced to the lytic phase, isolated, and lysed with NP-40. A subset of these cells were treated sequentially with the restriction endonuclease *Mlu*I and proteinase K. *Mlu*I cleaves p554-based concatemers multiple times. Cells lysed with NP-40 but not treated with *Mlu*I showed a strong band at the hexamer of p554 (172.8 kbp) with no detectable concatemers shorter than the hexamer (data not shown). Most of the detected DNA remained at the top of the gel either because it consisted of long concatemers or because it was retained as a DNA-protein complex. Cells treated with *Mlu*I and proteinase K yielded faint signals at the positions of pentamers, tetramers, and trimers of p554 but no detectable hexamers. The large DNA species found at the top of the gel in the absence of endonuclease digestion were cleaved to lengths of 30 kbp or less by *Mlu*I and represented most of the detected signal. These results are consistent with the analyses of intracellular p588 concatemers (Fig. 4). In both cases, a discrete range of concatemers of lengths shorter than that packaged most efficiently by the virus were found to be enriched within the cell.

B95-8 DNA is approximately 165 kbp in length. Viral particles which contained the DNA of the B95-8 helper virus copurified with the particles containing packaged p588 DNA in these experiments. Stripping and reprobing these blots provided a direct measure of the size of the viral genome. The results of this experiment, in which B95-8 and prominent p588 derivatives were detected (Fig. 5), indicate that the size of the B95-8 genome is approximately 165 kbp in length, or about 10

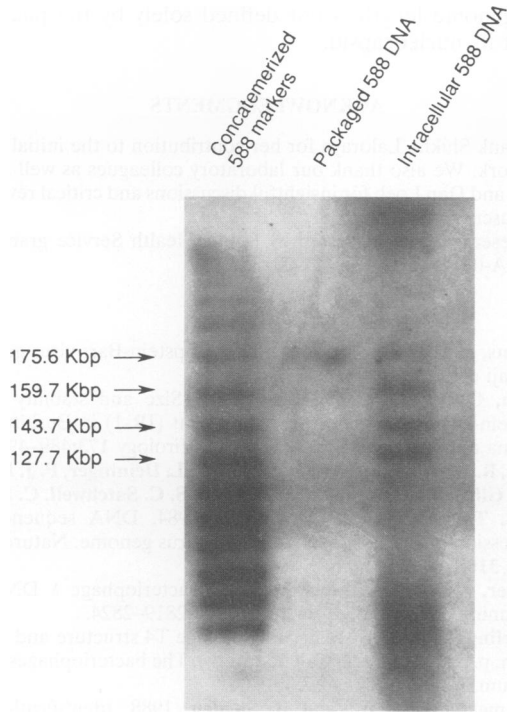

FIG. 4. The distribution of p588-based concatemers in cells supporting the lytic phase of EBV differs from the distribution of concatemers found in mature virion particles. Shown is an autoradiogram of DNAs isolated from virions (Packaged 588 DNA) and from intact cells (Intracellular 588 DNA). The molecular mass standard is a series of p588 concatemers generated by ligation. The first fully visible concatemer of the molecular mass standard is the dimer of p588. Cells used to isolate packaged p588 DNA and the cells used to isolate intracellular p588 DNA were from the same culture. The detectable signals shown in the lane representing packaged p588 DNA represent the 11-mer (176 kbp) and the 10-mer (160 kbp) (Fig. 2). The signals detectable in the lane containing intracellular p588 DNA represent the 11-mer (176 kbp), 10-mer (160 kbp), 9-mer (144 kbp), and 8-mer (128 kbp) of p588. It is important to note that the signals derived from intracellular DNAs ranging from 128 to 176 kbp in length are roughly equal in intensity.

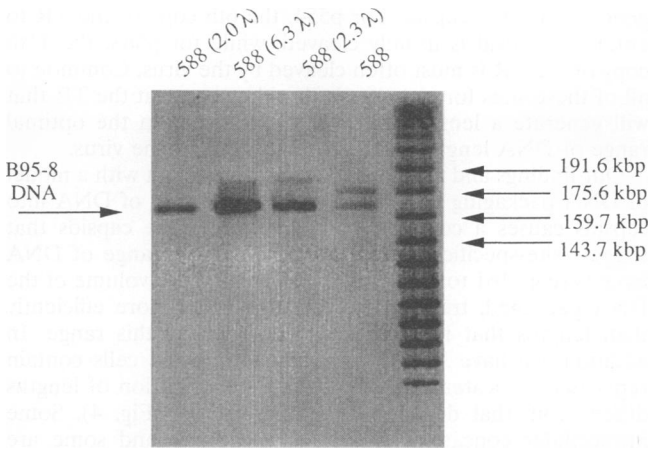

FIG. 5. The length of the B95-8 genome is approximately 165 kbp. A blot containing DNA isolated from experiments using p588, p588 (2.0 λ), p588 (2.3 λ), and p588 (6.3 λ) was stripped and reprobed for B95-8 DNA. The linear B95-8 genome migrated at approximately 165 kbp in length and is denoted by the arrow. The minor bands detected in the blot result from the hybridization of p588-based DNA to the probe. Some of the bands generated from p588-based plasmid DNA are of lengths coincident with the length of B95-8 [e.g., p588 (2.3 λ) (nonamer), 165 kbp], thereby increasing the signal in and near the region of the B95-8 DNA.

kbp shorter than the original estimated length of the B95-8 genome based on its sequence (3).

DISCUSSION

We have constructed plasmid derivatives of EBV that are packaged as concatemers of various lengths by an EBV helper virus. These plasmids contain all of the *cis*-acting elements necessary to be maintained, amplified, and packaged during the latent and lytic phases of the viral life cycle in B lymphoblasts. Some of the plasmids also contain various lengths of lambda DNA, so that the final range of DNA lengths of the five plasmids used in these experiments extended from 16 to 28.8 kbp. The plasmids were introduced into EBV-positive B95-8 cells, which were subsequently induced to support the lytic phase of the viral life cycle. During the lytic phase of EBV's life cycle, viral DNA replicates via generation of concatemeric replicative intermediates that are subsequently cleaved and packaged into virion particles (6, 7). We measured the lengths of the concatemeric DNAs derived from the introduced plasmids packaged into mature extracellular virion particles.

One striking finding from these measurements is that although the lengths of DNAs packaged by the virus spanned from 28.8 to 183 kbp, 75% or more of the packaged concatemers derived from any one plasmid ranged only between 161 and 179 kbp in length (Fig. 3 and data not shown). One might expect this finding to reflect the length distribution of DNA concatemers in the cell during packaging, but it does not. For example, p588-based concatemers with lengths of 127.7 and 143.7 kbp are abundant in the cell during lytic replication and yet are only rarely packaged (Fig. 4). These two observations together indicate that EBV packages DNA as a function of the length of the DNA being packaged.

Our findings place constraints on any model developed to describe EBV packaging in particular and herpesviral packaging in general. Viruses that use a headful packaging mechanism to delimit DNA packaged into capsids, such as bacteriophage T4, generate viral genomes that vary in length by approximately 1% (5). If we define the B95-8 strain as our archetype, then EBV preferentially packages DNAs from −2% to +10% of the archetypal length, a range much greater than that packaged by T4. The length of DNA is not the only determinant for packaging by EBV, because the TR are necessary for the cleavage and packaging of DNA by EBV (7). The TR alone do not define sites of DNA cleavage by EBV. Monomers of p588 and its derivatives, which would be generated by cleavage at each TR, were not detected as major signals either in mature virion particles or in the infected cells.

These observations indicate that both the presence of the TR and the length of the DNA being packaged affect the efficiency of DNA encapsidation by EBV. If we assume that the viral DNA is cleaved during its encapsidation, as is thought to be true for phage lambda (4), then the above-described notion of two required signals is strengthened by recognizing that the number of copies of the TR that pass into the viral capsid assembly prior to cleavage varies for each of the plasmids studied. For the B95-8 strain of EBV, cleavage occurs at the first complete TR encountered after packaging of the

genome into the capsid. For p554, the 6th copy of the TR to enter the capsid is usually cleaved, while for p588, the 11th copy of the TR is most often cleaved by the virus. Common to all of these sites for cleavage is that they occur at the TR that will generate a length of DNA that falls within the optimal range of DNA lengths efficiently packaged by the virus.

Our findings and interpretations are consistent with a model of DNA packaging by EBV in which packaging of DNA into capsids causes a conformational change in the capsids that triggers site-specific cleavage of the DNA. A range of DNA lengths (e.g., 161 to 179 kbp), which reflects the volume of the DNA packaged, triggers this cleavage event more efficiently than lengths that fall either above or below this range. In addition, we have shown that lytically infected cells contain replicated, concatemeric DNAs whose distribution of lengths differs from that detected in mature virions (Fig. 4). Some intracellular concatemeric DNAs are longer and some are shorter than the predominant species present in the mature virions. The smaller intracellular concatemers may be associated with immature capsids because they are resistant to digestion with the endonuclease *Mlu*I. One possibility is that these cleaved products either inhibit the maturation of the capsids in which they are packaged or are cleaved in capsids incapable of maturation. One caveat must be stressed in proposing any model to explain encapsidation of EBV DNA in general and our observations in particular. The concentration of EBV DNA in the nucleocapsid of a mature virion is on the order of 300 mg/ml. How this DNA is condensed such that it can be contained within the nucleocapsid is unknown, and this ignorance is likely to cloud any model proposed for packaging EBV DNA.

It was unexpected that the genome of the B95-8 strain of EBV measured approximately 165 kbp in length. This length differs from that previously predicted, 172 kbp, which was based on the sequence of B95-8 DNA compiled from the sequencing of M13 subclones and on an earlier estimation of lengths of fragments of B95-8 DNA separated electrophoretically in agarose gels (3). Our experiments provide a direct measure of the length of the B95-8 genome. The discrepancy between the length measurements is likely to result from differences in the numbers of *Bam*W (3.1-kbp) repeats, predicted by Baer et al. to be 11.6 in number (3) and measured by Allan and Rowe to be 8.6 (2). Within experimental error, a length of 165 kbp would be consistent with the fewer repeats measured by Allan and Rowe (2). It is possible that our measurements of the length of B95-8 DNA, as well as the lengths of the p588-based plasmids, are biased by anomalous migration in our gels. We think this possibility unlikely both because the measurements of Allen and Rowe are consistent with ours and because λ DNA molecular mass standards and the concatemers of the p588-based plasmids as well as p554 migrated as predicted from the lengths of their monomers.

According to our measurements, the B95-8 genome could expand by approximately 10% of its original length and still be packaged efficiently. The fact that the B95-8 genome has maintained a consistent length makes it an example of a virus whose genome length is not defined solely by the packaging limits of its nucleocapsid.

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