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Translocation of Nicked but not Gapped DNA by the Packaging Motor of Bacteriophage phi29

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The biomolecular mechanism that the double-stranded DNA viruses employ to insert and package their genomic DNA into a preformed procapsid is still elusive. To better characterize this process, we investigated packaging of bacteriophage phi29 DNA with structural alterations. phi29 DNA was modified *in vitro* by nicking at random sites with DNase I, or at specific sites with nicking enzyme N.BbvC IA. Single-strand gaps were created by expanding site-specific nicks with T4 DNA polymerase. Packaging of modified phi29 DNA was studied in a completely defined *in vitro* system. Nicked DNA was packaged at full genome length and with the same efficiency as untreated DNA. Nicks were not repaired during packaging. Gapped DNA was packaged only as a fragment corresponding to the DNA between the genome terminus and gap. Thus the phi29 DNA packaging machinery tolerated nicks, but stopped at gaps. The packaging motor did not require a nick-free DNA backbone, but the presence of both DNA strands, for uninterrupted packaging.

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Introduction

Double-stranded (ds) DNA viruses assemble by inserting their linear genome into a preformed protein shell. Several models[1–9] were suggested to explain this DNA packaging process that is accomplished by an ATP hydrolyzing motor complex, but, at the molecular level, the biomechanical mechanism remains unknown. It has been speculated that there is a common basic packaging mechanism that is shared by the dsDNA bacteriophages,[3] and possibly also by dsDNA viruses including herpesvirus, poxvirus and adenovirus. The incentive to study bacteriophage DNA packaging is not only to shed light on a fundamental biomechanical process, but also to provide clues for possible novel targets for antiviral therapy,[10–13] and to explore this class of strong and highly processive motors[14] for possible applications in nanobiotechnology.[15–18]

Bacteriophage phi29 has become a model for viral

linear dsDNA packaging studies. It is a well-studied phage (see Meijer *et al.*[19] for a review) with a comparatively small 19,285 bp genome. The DNA carries one copy of virus-encoded protein gp3 covalently attached to each 5′ end (DNA-gp3).[20] Unlike the DNA of other well-characterized phages, including lambda, T3, T4 and others, it is not packaged as a concatemer but as a unit length entity. A notable feature of the phi29 family of phages is that they involve an RNA molecule that is essential for DNA packaging.[21] A highly efficient *in vitro* DNA packaging[22] and phage assembly system based exclusively on purified recombinant or synthetic components[23] is available. This system was used to collect a wealth of information on phi29 DNA packaging (see Grimes & Anderson[24] and Guo[25] for reviews), including characterization and force measurement of the motor by a single-molecule approach.[14] The phi29 DNA packaging motor hydrolyzes one ATP for the translocation of two DNA base-pairs.[26] The crystal structure of the connector, a central component of the DNA packaging motor, was solved to high resolution.[7,8,27] Detailed structural studies of the complete phage, the procapsid and the DNA packaging motor were also performed by cryo-electron microscopy.[7,28–30]

We sought to improve the understanding of the

biomolecular mechanism of phi29 DNA packaging by studying DNA with single-strand gaps or breaks (nicks) as a substrate for the motor. A previous report showed that DNA crosslinks or nicks do not interfere with DNA packaging in bacteriophage T3.[31] The authors also speculated that T3 could not package single-stranded DNA, but they did not specifically indicate how they obtained this result. The DNA packaged in mature infectious virions of several phages was found to contain structural alterations. In the genome of bacteriophage T5, single-chain breaks occur with variable frequencies at fixed sites in the genome.[32,33] Nicks and site-specific nicks were also detected in packaged bacteriophage T7 DNA, and it was speculated that premature activity of terminase during packaging is responsible for these nicks.[34] Some less well characterized bacteriophages seem to possess considerable tolerance for structural abnormalities in the DNA they package. Mycobacteriophage I3 was found to contain several single-strand gaps in its packaged dsDNA genome.[35] *Rhodopseudomonas sphaeroides* bacteriophage RSI DNA is heterogeneous in length and contains nicks or gaps.[36] Extended gaps were found in DNA packaged by *Erwinia herbicola* phage ErhI.[37] Alterations in the structure of DNA that block packaging were reported for mutants of bacteriophage T4. Mutants in gene 49 cannot resolve Holliday junctions, and these recombination intermediates block packaging.[38] Nicks were reported to cause a reversible block to the completion of T4 packaging in ligase-deficient mutants.[39] These stalled procapsids can be rescued by restoring ligase.[40] Other mutations in T4 early genes, including the gene for topoisomerase II subunit gp39, were also reported to cause aberrant DNA structures that cannot be translocated.[41] The effects of DNA heterologies on bacteriophage packaging were studied for phage lambda.[42] The authors annealed DNA from *cI*$^+$ phage with DNA from phages carrying small insertion or deletion mutations in the *cI* gene. They found that heterologies of 19 bp but not 26 bp could be packaged efficiently.

Bacteriophage phi29 can package restriction fragments of its genome efficiently, provided that the terminal protein gp3 is present. In extracts of infected cells, packaging initiates from the left end of the genome,[43] but in the defined *in vitro* DNA packaging system that we used in this study, packaging can also start from the right end.[44] Extension of the phi29 genome is compatible with packaging.[14] Beyond these observations, packaging of altered DNA has not been studied for bacteriophage phi29, and packaging of DNA with single-strand gaps has not, to our knowledge, been studied for any of the tailed dsDNA bacteriophages.

In several previously proposed DNA packaging models, the helical nature of *B*-DNA plays a fundamental role. An extrapolation of these models would suggest that single-stranded DNA cannot be packaged. phi29 DNA-gp3 was previously shown to form lariats that are supercoiled by the DNA packaging ATPase gp16.[45] The process was suggested to be a maturation step that makes phi29 DNA-gp3 competent for packaging. This work is related to our current study because nicks in the lariat loops would relax supercoils. Here, we modified phi29 DNA-gp3 *in vitro* to introduce nicks or gaps. We packaged modified DNA using the defined *in vitro* packaging system,[22] and we analyzed DNase I-protected (i.e. packaged) DNA by agarose gel electrophoresis. We found that phi29 DNA containing nicks was packaged at whole genome length and without loss of packaging efficiency, but packaging stopped at gaps in the DNA.

Results

Packaging of nicked DNA

Identical aliquots of phi29 DNA-gp3 were treated with increasing amounts of DNase I, and analyzed by agarose gel electrophoresis in Tris–acetate/EDTA (TAE) and in alkaline buffer (Figure 1(a) and (c)). In the TAE gel, a sample of a 2 μg aliquot of DNA treated with 1 pg of DNase I looked the same as undigested DNA, whereas treatment with 100 pg of DNase I led to the degradation of phi29 DNA-gp3 to fragments of 5 kb and smaller. Intermediate concentrations of DNase I caused partial degradation. Since the DNase I digestion was performed in a Mg^{2+}-containing buffer, the enzyme was expected to nick single strands of DNA at random sites rather than cut both strands at the same site.[46] To confirm the presence of nicks, samples of the preparations were denatured to single-stranded DNA, and analyzed by alkaline agarose gel electrophoresis (Figure 1(c)). The single-stranded fragments were shorter than the corresponding double-stranded fragments, thus revealing that the DNA was nicked. These same DNA preparations were used as substrate for packaging in a defined *in vitro* system[22] (Figure 1(b)). phi29 procapsids with bound prohead RNA, the packaging ATPase gp16, and ATP were mixed with the DNA, and after incubation, non-packaged DNA was digested with DNase I. Packaged DNA was ejected from procapsids and analyzed by agarose gel electrophoresis. DNA treated with up to 10 pg of DNase I per 2 μg aliquot was packaged with the same efficiency as undigested DNA (Figure 1(b) lanes 1–4). Fragments of more strongly degraded DNA, where no full-length DNA remained in the preparation, were also packaged (Figure 1(b) lanes 5 and 6). The phi29 DNA packaging motor did not stop at nicks, and nicks in the DNA did not seem to decrease the overall DNA packaging efficiency. For example, the DNA sample treated with 10 pg of DNase I showed single-stranded fragments predominantly in the size range of 3–8 kb in the alkaline gel (Figure 1(c) lane 4). Consequently, a complete 19,285 bp genome contained between five
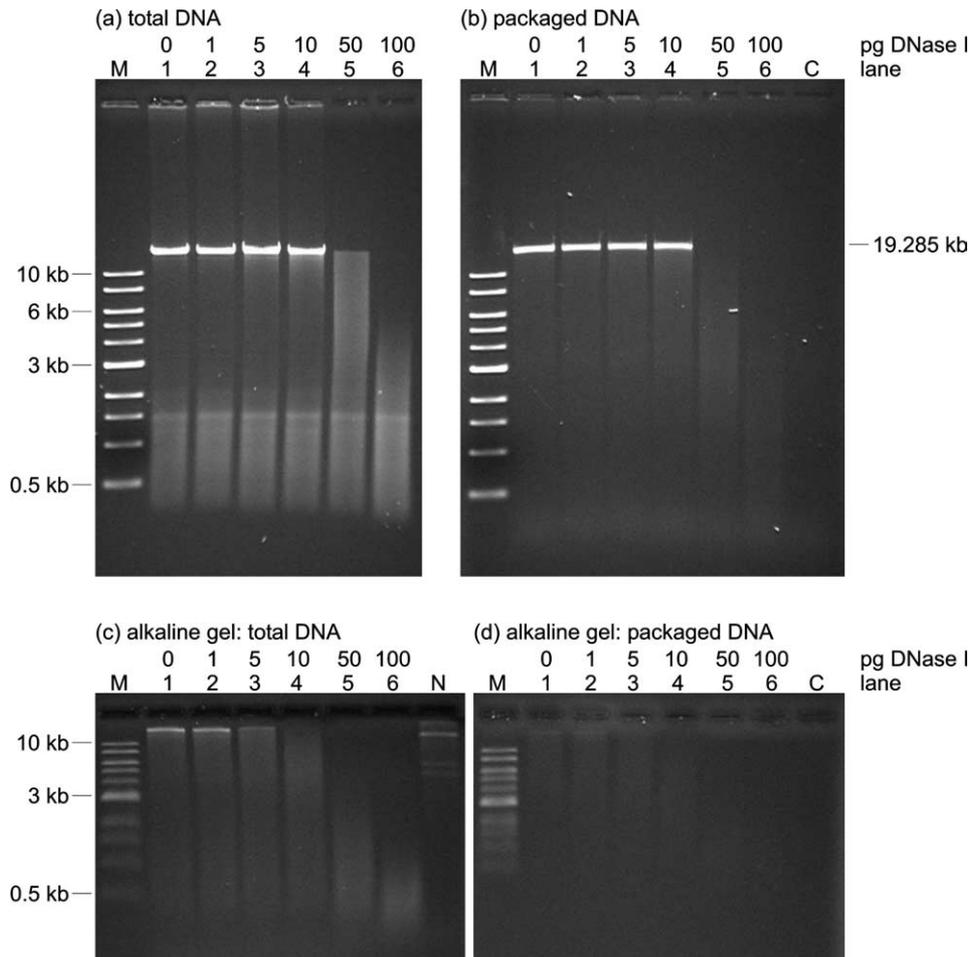
Figure 1. Packaging of nicked bacteriophage phi29 DNA. Identical aliquots of phi29 DNA-gp3 were treated with 0–100 pg (indicated above lanes) of DNase I. M, molecular mass marker. (a) This shows 2 µg of DNA after nicking. (b) Identical amounts of the same DNA preparations as in (a) were packaged in the phi29 defined *in vitro* DNA packaging system, and non-packaged DNA was digested with DNase I. C is a control where ATP was omitted from the packaging reaction to show that an identical amount of phi29 DNA-gp3, if not packaged and therefore not protected inside the procapsid, was completely digested. (c) Identical amounts of the same DNA preparations as in (a) were denatured to single-stranded DNA and separated on an alkaline gel. N is a control of phi29 DNA-gp3 nicked with N.BbvC IA. Due to the presence of nicks, the single-stranded DNA fragments were shorter than the double-stranded fragments of the respective preparations. (d) DNA packaged as in (b) was denatured to single strands and separated in an alkaline gel. Even though the amount of packaged DNA was at the limit for detection by alkaline agarose gel electrophoresis and ethidium bromide staining, the gel shows that, for respective preparations, the molecular mass range of single strands of (c) total and (d) packaged DNA were similar.

and 13 nicks on average, yet it was packaged with the same full efficiency as undigested DNA (Figure 1(b) lane 4). For the strongly degraded preparation digested with 50 pg of DNase I, DNA fragments ranging predominantly from 2 kb to 10 kb were packaged (Figure 1(b) lane 5), yet the alkaline gel showed that single strands were no longer than 2 kb (Figure 1(c) lane 5), and therefore, packaging must have proceeded over several nicks. The amount of DNA that was packaged in one *in vitro* packaging reaction was close to the detection limit of alkaline agarose gel electrophoresis and ethidium bromide staining (Figure 1(d)). However, compared to the respective preparation of total DNA (Figure 1(c)), packaged DNA showed the same range of molecular masses of denatured single-stranded DNA fragments.

Packaging of gapped DNA

We introduced single-strand gaps in phi29 DNA-gp3 by employing the 3′ to 5′ exonuclease activity of T4 DNA polymerase to expand nicks to gaps. First, two site-specific nicks were cut with enzyme N.BbvC IA, which has one site on each strand of phi29 DNA (Figure 2). The exonuclease reaction was performed in the presence of dTTP to make the enzyme's polymerase activity override its exonuclease activity at the 3′ ends of the phi29 genome, which terminate with three consecutive deoxythymidylate residues. Therefore, the DNA ends were supposed to be protected. The gap expansion reaction may also be expected to stall at Ts in the sequence. At the 5′ side of the N.BbvC IA site in the top strand at base 5883, there are Ts at a distance of
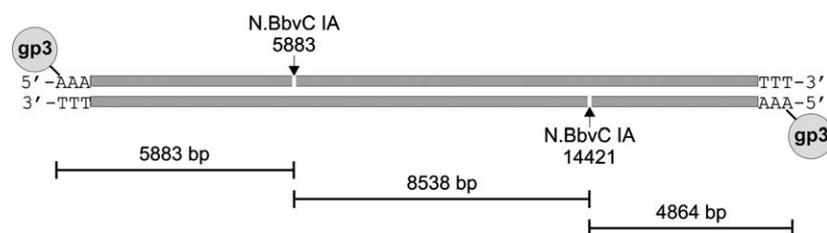
Figure 2. Schematic diagram of sites for nicking enzyme N.BbvC IA in phi29 DNA-gp3 (19,285 bp). gp3 is the terminal protein that is covalently linked to each 5′ end. Since the expansion of nicks to gaps at the N.Bbvc IA sites by the 3′ to 5′ exonuclease activity of T4 DNA polymerase was performed in the presence of dTTP, the 3′ ends of the phi29 genome were protected. Packaging initiates predominantly from the left end of the genome.

3, 8, 25 and 33 bases, and there are three consecutive Ts 35–37 bases from the nicking site. The other N.BbvC IA site, in the bottom strand at base 14,421, has Ts at 11, 20, 23, 29, 32, and TTs at a distance of 25–26, 34–35 and 41–42 bases in the 5′ direction from the nicking site (for the sequence in these two regions of the phi29 genome see Vlcek & Paces[47]). Agarose gel electrophoresis after the gapping procedure showed that N.BbvC IA had a small residual BbvC I restriction enzyme activity, since it cut both DNA strands at the nicking site in a small percentage of DNA (Figure 3(a) lanes 2 and 3). Expansion of nicks to gaps was confirmed by digestion with mung bean nuclease, which preferentially digests single-stranded DNA (Figure 3(b)).[48] Only the DNA preparation treated with both N.BbvC IA and T4 DNA polymerase, i.e. the gapped DNA, was cleaved to a characteristic pattern of fragments of 8.5, 5.9 and

4.8 kb. These fragment sizes match the expected cleavage at the single-strand gap sites. Identical amounts of the four DNA preparations (the same amounts as used for electrophoresis of total DNA and mung bean nuclease digestion (Figure 3(a) and (b))) were subjected to *in vitro* packaging in the same defined system[22] that was used for packaging of nicked DNA. DNA preparation 2, which was treated with nicking enzyme but not T4 DNA polymerase, was packaged with the same efficiency as undigested DNA (Figure 3(c) lanes 1 and 2). T4 DNA polymerase-treated DNA was packaged with reduced efficiency, but at full length (Figure 3(c) lane 4). In contrast, the DNA treated with both nicking enzyme and T4 DNA polymerase, i.e. the DNA containing single-strand gaps, was not packaged at full genome length. The packaged DNA formed a band at 5.8 kb. This fragment size matches the DNA between the left end of the phi29
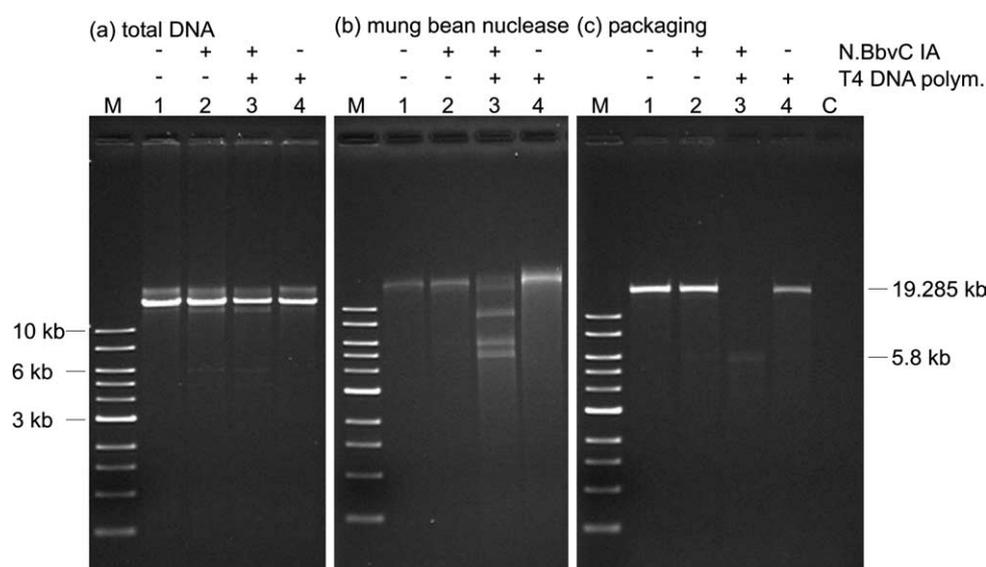

Figure 3. phi29 packaging of DNA with single-strand gaps. The gaps were made by expanding site-specific N.BbvC IA nicks with T4 DNA polymerase. All four DNA preparations were made in parallel under identical conditions, except that one or both enzymes, as indicated, were omitted in controls. Identical amounts (2 μg) of the same DNA preparations as in (a) were treated with mung bean nuclease (b), or subjected to *in vitro* packaging (c). Mung bean nuclease treatment resulted in the pattern of fragments expected from cleavage at the single-strand gap sites in sample 3. Whereas full genome-size fragments of the control DNA preparations were packaged, only a 5.8 kb fragment of the DNA with gaps (sample 3) was packaged. C is a control where ATP was omitted from the packaging reaction to show that an identical amount of non-packaged phi29 DNA-gp3 was completely digested.

genome and the first gap site at base 5883. The fragment indicated that DNA packaging stopped at the gap in the DNA.

Discussion

We investigated whether bacteriophage phi29 can package DNA containing nicks or gaps. The DNA modifications were made *in vitro*, and DNA packaging was studied by agarose gel electrophoresis to identify the packaged DNA after packaging in a defined *in vitro* system.[22] We found that nicks in the DNA had no effect on packaging. DNA translocation into the viral procapsid did not stop at nicks, and the overall packaging efficiency was not reduced. The alkaline gels showed that phi29 packaging did not seem to prefer intact over nicked DNA, and that the nicks were not repaired during the packaging process. A similar result was previously reported for bacteriophage T3.[31] However, it has been suggested that a nick is a structural alteration of DNA that causes stalling of the bacteriophage T4 DNA packaging motor.[39,40] Our result, therefore, indicates a difference in findings between phi29 (and T3) and T4 DNA packaging motors. It should be noted that in contrast to the present study and the previous study on T3, the conclusion regarding packaging of nicked T4 DNA was not derived from a defined *in vitro* packaging system, but from the study of ligase-deficient mutants. Consequently, it cannot be ruled out that the structural alterations of DNA that stalled DNA packaging in T4 were actually more complex than just nicks. It has been found that phi29 DNA-gp3 undergoes a maturation process that makes it competent for packaging. This process involves lariat formation and supercoiling of the DNA ends by proteins gp3 and gp16.[45] Since a nick would relax supercoils, our data suggest that either a short supercoiled lariat at the DNA ends is sufficient, or possibly that the previously observed supercoiling is a concomitant phenomenon rather than an essential requirement of DNA packaging.

We found that phi29 DNA with single-strand gaps was not packaged at full genome length. The gaps had a non-uniform size in the range of tens of bases. Only the DNA fragment between the left end of the genome and the first gap was packaged (Figure 3(c)). This result suggests that a single-strand gap in the DNA is a structural alteration that causes the packaging motor to stop. Strictly speaking, our data do not allow an unambiguous conclusion on whether packaging stopped at the beginning or the end of the gap. However, since the packaged DNA did not form a sharp band at 5883 bp, but rather a diffuse band seemingly derived from a population of heterogeneous fragments, we tend to infer that packaging stalled at the beginning of the gap. By using the 3′ to 5′ (i.e. towards packaging initiation site at genome end, see Figure 2) exonuclease activity of T4 DNA polymerase, we created a distribution of gap sizes

rather than a certain number of missing nucleotides. The gaps had defined ends at the nicking sites (5883 and 14,421 bases from the left end), but the beginning of the gaps, 5′ of the nicking sites and closer to the genome ends, were not uniform. Therefore, the appearance of a diffuse band rather than a sharp 5883 bp band indicated that packaging likely stopped at the beginning of a gap, which would mean that single-stranded DNA cannot be packaged. Since the gaps that we produced did not have a defined size, we cannot rule out that the motor can package DNA with small gaps of just one or a few nucleotides.

Compared to unmodified or nicked DNA, the DNA treated with T4 DNA polymerase was packaged with decreased efficiency. We employed the enzyme's 3′ to 5′ exonuclease activity to convert site-specific nicks to single-strand gaps. The gapping reaction was performed in the presence of dTTP to protect the 3′ ends of phi29 DNA, which terminate with three consecutive deoxythymidylate residues, by the enzyme's polymerase activity. The reduction in DNA packaging efficiency was likely due to incomplete blocking and partial degradation of the DNA ends. When we tested the gapping reaction at milder conditions, including lower temperature, higher dTTP concentration or shorter incubation time, we found that the packaging efficiency was higher, but gapping was incomplete (data not shown). Under these conditions we observed additional packaged fragments: a 4.8 kb fragment corresponding to the part of the genome between the right end and the second gap, a ∼14 kb fragment corresponding to packaging through the first but not the second site, and a full-length genome fragment. It has previously been reported that in the *in vitro* DNA packaging system that we used, in contrast to packaging in infected cell extracts,[43] the phi29 genome can also be packaged from the right end.[44] We confirmed this result by packaging restriction fragments of phi29 DNA-gp3 (not shown). However, under the conditions required for converting all site-specific nicks to gaps, which resulted in reduced packaging efficiency, we only saw the left end fragment of the genome packaged. The observation that N.BbvC IA-nicked DNA is packaged like untreated DNA is in agreement with our findings on DNA nicked at random sites with DNase I.

The possibility to stop DNA packaging at specific gap sites in the DNA may find applications in further work on phi29 DNA packaging. An alternative method of preparing stalled packaging intermediates with a partially filled procapsid and partially protruding DNA is based on γ-S-ATP. This non-hydrolyzable analog of ATP was included at low concentrations in packaging reactions with ATP,[49] or used at higher concentrations to stop packaging reactions 30 seconds after initialization with ATP.[14] Since it has thus far not been possible to prepare phi29 packaging initiation complexes that could be used to synchronize DNA packaging as with phage T3,[50] the γ-S-ATP method of preparing

stalled complexes is inefficient. Partial packaging of gapped DNA-gp3 may be a way to prepare and isolate partially packaged procapsids at high yield. Such packaging intermediates would be desirable for further structural or functional studies on DNA packaging, for instance to map the location of the packaging ATPase gp16 by cryo-EM, to study the arrangement of DNA in capsids, or to investigate the exit of scaffolding protein gp7 and the structural transition of the capsid to the mature, more angular form. Since the presently reported approach for creating gapped DNA caused a reduction in packaging efficiency, high yield preparation of partially packaged procapsids may require an alternative gapping method. Such a method could, for instance, be based on mutant DNA with two nicking sites in close proximity, so that a short oligonucleotide could be removed by mild heating. Another interesting aspect of stopping DNA packaging at gaps is its potential in the proposed application of biomolecular motors in nanobio-technology.[16,17] The possibility to operate and stop a DNA packaging motor at a precise point, defined by the location of a single-strand gap in the DNA, is probably unique and may be desirable for possible future integration of biomolecular motors in nanoelectromechanical systems. Both for the context of applications and functional studies of DNA packaging, it would be interesting to test if stalled procapsids can be restarted by filling the gap.

We gained some insight into aspects of the biomolecular mechanism of bacteriophage phi29 DNA packaging by modifying the DNA. The modification that could be packaged (nick) and the alteration that stopped packaging (gap) can be interpreted as limits that help define the DNA structural requirements for the packaging motor. In several previously proposed DNA packaging models, the helical nature of *B*-form DNA plays a crucial role. Our finding that packaging stalled at gaps, where the helical structure was interrupted, fits in with these models. One intact DNA strand does not seem to be sufficient for phi29 DNA packaging, but the packaging machinery, whether it acts by active power strokes[6–8] or as a ratchet,[9] seems to require the presence of both strands as a minimum essential condition. Our finding that nicks in the DNA had no effect on packaging does not favor models in which DNA supercoiling plays a major role. It would be interesting to test packaging of nicked and gapped DNA with defined *in vitro* packaging systems of other phages. Even though it is frequently assumed that there is a common basic DNA packaging mechanism that all tailed dsDNA phages use, there may be substantial differences. An indication for such differences is that single-strand gaps were previously found in the packaged dsDNA genomes of certain bacterio-phages.[35,37] In addition to providing new information indicating that phi29 DNA packaging will stop at single-strand gaps but not nicks, our work may lay the groundwork for new approaches to bacteriophage DNA packaging studies and appli-cations, based on the use of gaps to prepare stalled DNA packaging motor complexes.

Materials and Methods

Preparation of phage components for *in vitro* packaging

phi29 DNA-gp3, gp16, and phi29 procapsids were prepared as described.[22]

Preparation of nicked phi29 DNA-gp3

Identical aliquots of 2 μg of phi29 DNA-gp3 in 40 μl of buffer (50 mM NaCl, 10 mM Tris–HCl (pH 7.9), 10 mM MgCl$_2$, 1 mM dithiothreitol) containing 100 μg/ml of BSA and ten units of HaeIII (New England Biolabs; HaeIII does not cut phi29 DNA[51] and was used to fragment *Bacillus subtilis*-derived DNA in the phi29 DNA-gp3 preparation) were prepared. DNase I (Sigma D4527, 2000 Kunitz units/mg) was serially diluted in the same buffer containing BSA. One microlitre of DNase I (100; 50; 10; 5; 1; 0 ng/ml) was added per aliquot, and all samples were incubated for one hour at 22 °C. Then 2 μl of 500 mM EDTA, 40 μl of 2 M NaCl and 80 μl of 20% (w/v) polyethylene glycol 8000 (Sigma) were added, and the aliquots were incubated for 14 hours at 0 °C for precipitation.[52] The DNA was recovered by 30 minutes centrifugation at 14,000 rpm in a microcentrifuge at 4 °C, and the pellets were dissolved in 10 μl of TE buffer (10 mM Tris–HCl (pH 7.6), 1 mM EDTA).

Preparation of gapped phi29 DNA-gp3

Four identical aliquots of 2.4 μg of phi29 DNA-gp3 in 25 μl of buffer (50 mM NaCl, 10 mM Tris–HCl (pH 7.9), 10 mM MgCl$_2$, 1 mM dithiothreitol) were prepared. Two units of nicking enzyme N.BbvC IA (New England Biolabs) were added to aliquots 2 and 3, and all four samples were incubated at 37 °C for one hour. The volume was increased to 50 μl of the same buffer containing (final concentration) 400 μM dTTP and 100 μg/ml of BSA. Then 2.4 units of T4 DNA polymerase (New England Biolabs) were added to samples 3 and 4, and all samples were incubated at 37 °C for 28 minutes. Since the 3′-terminal sequence of both strands of phi29 DNA-gp3 is TTT, dTTP was included in the reaction to limit the 3′-5′ exonuclease activity of T4 DNA polymerase to the nicks, while the DNA ends are protected by the enzyme's polymerase activity. All samples were brought to a final dTTP concentration of 1 mM, incubated at 37 °C for two more minutes, followed by addition of 2 μl of 500 mM EDTA. The DNA was precipitated with polyethylene glycol as described above and dissolved in 12 μl of TE buffer.

Mung bean nuclease digest

Two microlitres of DNA from the gapping procedure described above were incubated with 4.5 units of mung bean nuclease (Promega) in a total volume of 11 μl of mung bean nuclease buffer (Promega) containing 10% (v/v) glycerol and 100 μg/ml of BSA for 30 minutes at 37 °C. Reactions were stopped with 2 μl of 500 mM EDTA.

phi29 DNA packaging

phi29 DNA-gp3 was packaged *in vitro* as described.[22] One standard packaging reaction containing 1 μl of procapsid (about 1×10^{13} procapsids/ml, isolated from mutant phage-infected *Bacillus subtilis*), 6 μl of gp16 (about 10 mg/ml) and 1 mM ATP was used to package 2 μg of DNA from the nicking or gapping reaction. The packaging reactions with DNA from the gapping procedure were complemented with 100 μM dTTP to stop the exonuclease activity of residual T4 DNA polymerase on DNA ends. After incubation for 70 minutes at 22 °C, 1 μl of DNase I (1 mg/ml; Sigma) was added to degrade the DNA that had not been packaged. Aliquots of 2 μl of 500 mM EDTA were added 30 minutes later, and the DNA was ejected from procapsids at 75 °C for 20 minutes. RNA was degraded with 1 μl of RNase A (1 mg/ml, Sigma) for 30 minutes at 22 °C.

Agarose gel electrophoresis

DNA samples were prepared for agarose gel electrophoresis by incubation with 20 μg of proteinase K (Sigma) for one hour at 55 °C to degrade the terminal protein gp3.[20] Electrophoresis in 0.7% (w/v) agarose gels in TAE buffer (40 mM Tris–acetate, 1 mM EDTA) and staining with ethidium bromide were performed according to standard procedures.[48] For alkaline gel electrophoresis, proteinase K-digested DNA was incubated in 200 mM NaOH for 30 minutes at 22 °C. A 0.7% agarose gel in 50 mM NaOH, 1 mM EDTA was prepared, run and stained with ethidium bromide according to standard procedures.[48]

Acknowledgements

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Edited by J. Karn