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Only one pRNA hexamer but multiple copies of the DNA-packaging protein gp16 are needed for the motor to package bacterial virus phi29 genomic DNA

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Abstract

A common feature in the maturation of linear dsDNA viruses is that the lengthy viral genome is translocated with remarkable velocity into a limited space within a preformed protein shell using ATP as motor energy. Most biomotors, such as myosin, kinesin, DNA-helicase, and RNA polymerase, contain one ATP-binding component that acts processively. An examination of the well-studied dsDNA viruses reveals that DNA packaging motors involve two nonstructural components. Which component of the motor is the integrated processive factor to turn the motor has not been identified. In bacterial virus phi29, these two components consist of a gp16 protein and an RNA molecule called pRNA. We have previously predicted and recently confirmed that gp16 binds ATP. It is generally believed that gp16 serves as an ATP-binding and processive component to drive the motor. In this article, phi29 DNA-packaging intermediates were purified in quantity and examined to differentiate the role between gp16 and pRNA. It was found that the pRNA hexamer is an integral motor component, while gp16 is not stably bound. Only one pRNA hexamer, but multiple copies of gp16, were needed to accomplish DNA packaging. pRNA functions continuously during the entire DNA translocation process, suggesting that pRNA is a vital part of the DNA packaging motor.

Keywords: DNA-packaging; Nanomotor; RNA structure; Viral assembly; Proccessivity; ATPase; DNA-packaging intermediates; Viral assembly intermediates

Introduction

Extensive investigation on the assembly of dsDNA viruses documents a common feature: the lengthy viral genome is translocated quite rapidly into a confined area inside a preformed protein shell and packaged into a near crystalline density. This energetically unfavorable DNA packaging reaction is accomplished by a viral DNA packaging motor (Black, 1989; Earnshaw and Casjens, 1980; Guo, 1994). ATP hydrolysis supplies the energy to drive this otherwise unfavorable motion reaction. One ATP is needed to package two base pairs of DNA (Guo et al., 1987; Morita et al., 1993). An examination of dsDNA viruses reveals that DNA packaging motors involve two nonstructural components, at least one being a typical ATPase. In bacterial virus phi29, the two components are a protein, gp16, and an RNA, pRNA.

Bacterial virus phi29 is an ideal system for the study of the mechanism of DNA packaging due to its high efficiency of in vitro DNA packaging (Guo et al., 1986). All components needed to package phi29 DNA and to assemble infectious virions have been purified. The in vitro assembly system can convert a DNA-filled capsid into an infectious virion. With this efficient system, up to 10^9 PFU/ml of infectious virions can be assembled in vitro, while the omission of a single component results in no plaque formation (Lee and Guo, 1995). Thus, there is a system with eight orders of sensitivity for the analysis of the structure and function of each component, and for the elucidation of how

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the DNA-packaging motor functions. The structure of the connector, one of the components of the DNA packaging motor, has been solved by X-ray crystallography (Guasch et al., 2002; Simpson et al., 2000). The phi29 DNA packaging motor has been reported to be the strongest motor studied to date, with a stalling force of more than 50 pN and an initial speed of 100 bases per second under external load (Smith et al., 2001). The pRNA has been shown to form a hexamer that gears the DNA-packaging motor (Guo et al., 1998; Hendrix, 1998; Trottier and Guo, 1997; Zhang et al., 1998). A computer model of the pRNA hexamer and the motor complex has been presented (Hoeprich and Guo, 2002).

To ensure continuous motion of a motor, at least one component should act processively or continuously. Most motor proteins, including myosin (Inoue et al., 2002; Romberg and Vale, 1993), kinesin (Hackney, 1995; Schnitzer and Block, 1997), DNA-helicase (Pang et al., 2002; West, 1996), RNA polymerase (Harada et al., 2001; Wirtz et al., 1998), and the T_7 DNA-ejection motor (Molineux, 2001), involve one component that acts processively. Increasing evidence suggests that viral DNA-packaging motors are similar in some respects to DNA or RNA processing, riding, or sliding enzymes such as DNA-helicase, RNA polymerase, and the RNA transcription termination factor Rho (Guo et al., 1998; for review, see Guo, 2002). DNA-packaging components are apparently required to act continuously during DNA translocation. For example, the use of certain temperature-sensitive mutants of T_4 terminase can lead to cessation of DNA packaging and production of partially filled procapsids (Wunderli et al., 1977). Continuous action of λ-terminase is required for packaging (Becker et al., 1977). However, which component of the phi29 DNA packaging motor acts continuously has not been previously demonstrated; gp16 and pRNA are possible candidates. It is shown here that pRNA is the integral component in the phi29 DNA-packaging motor. This finding was made possible by a new approach that allowed for the isolation of higher quantities of phi29 DNA packaging intermediates.

Results

Isolation of DNA-packaging intermediates

Assembly of the infectious phi29 virion in vitro includes two steps: DNA packaging and virion maturation. Due to the rapidity of the DNA-packaging process, it has been very difficult to elucidate intermediates. Isolation of intermediates in quantity for biological assays has not been reported previously due to the lack of a sensitive method to identify them. However, with the aid of the highly sensitive phi29 in vitro assembly system, these intermediates have now been isolated.

Phi29 DNA packaging was performed in a mixture containing procapsid, gp16, pRNA, genomic DNA-gp3, ATP, and magnesium ion. The poorly hydrolyzable ATP analogue γ-S-ATP was used to block the DNA packaging for the isolation of phi29 DNA-packaging intermediates. When γ-S-ATP was present, DNA packaging was incomplete and part of the genome extended from the procapsid. The intermediates were separated from finished DNA-filled capsids, empty procapsids, or free DNA by sucrose gradient sedimentation. The finished DNA-filled capsids centered at fraction 8 of the gradient (Fig. 1), while smaller or lighter particles, such as free DNA, stayed near the top of the gradient. After sedimentation, the finished DNA-filled capsids and the DNA packaging intermediates in each fraction of the gradient were converted into matured infectious phi29 virions through the addition of maturation components (Fig. 1). When γ-S-ATP was not added into the DNA-packaging mixture, few DNA-packaged intermediates were detected. However, when 5% of the ATP was replaced by γ-S-ATP, DNA-packaging intermediates with slower sedimentation rates were produced (Fig. 1).

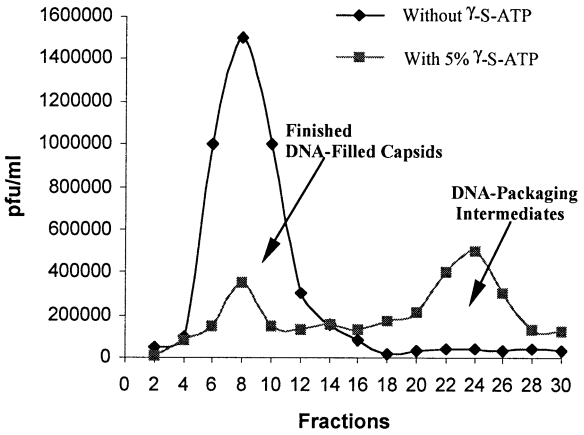

Fig. 1. Use of the poorly hydrolysable ATP analogue γ-S-ATP to produce DNA-packaging intermediates. After DNA packaging, the intermediates were separated from free DNA and the finished DNA-filled procapsids by 5–20% sucrose gradient. The intermediates in each fraction of the gradient were subsequently converted into infectious phi29 virion, expressed as plaque-forming unit per milliliter (PFU/ml) by the addition of components required for phi29 maturation. Sedimentation is from right to left (see Materials and methods).

Requirement of gp16 and pRNA for the formation of DNA packaging intermediates

The aforementioned isolation method of DNA-packaging intermediates was used to find out which components were necessary for the formation of DNA-packaging intermediates. After sucrose gradient sedimentation, DNA packaging intermediates were converted into infectious phi29 virion by the addition of the maturation components (Fig. 2). It was found that both gp16 and pRNA were needed for the formation of the intermediates. If either gp16 or pRNA was omitted from the assembly mixture, no finished DNA-

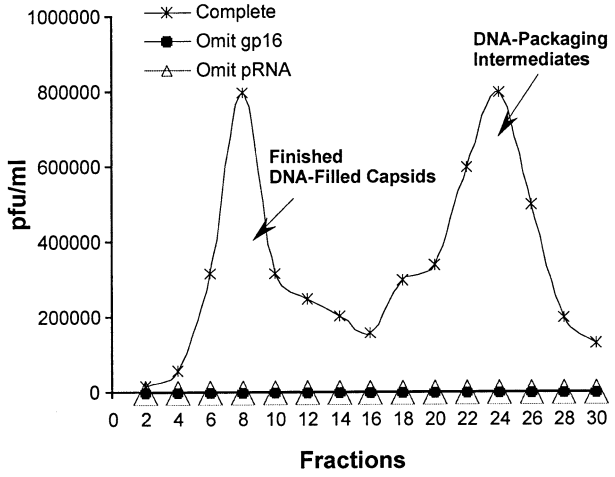

Fig. 2. Conversion of DNA-packaging intermediates into mature virions to demonstrate the requirement of pRNA and gp16 for the initiation of DNA packaging. "Omit gp16" or "Omit pRNA" indicates that during the first DNA packaging step, gp16 or pRNA, respectively, was omitted from the DNA packaging mixture. "Complete" indicates the inclusion of all components needed in the first DNA-packaging step. The components in each fraction of all gradients were subsequently converted into infectious phi29 virion by the addition of maturation components.

filled capsids or DNA-packaging intermediates were produced (Fig. 2).

Requirement of fresh gp16 for maturation of the intermediates

Isolated DNA-packaging intermediates were tested to find out which components are needed to complete the packaging process. ATP, gp16, and pRNA were added individually, or in combination, with maturation proteins into each fraction of a gradient that had separated filled capsids from intermediates. This procedure allows infectious virions to be made from filled capsids without further addition of gp16 or pRNA (Fig. 3). Conversion of the intermediates to filled capsids required adding gp16 (Fig. 3B). However, intermediates contained the necessary pRNA and no additional pRNA was needed to convert them into infections virion (Fig. 3A), indicating that the bound pRNA is sufficient for the continuation of the genome packaging.

Continuous function of pRNA during DNA translocation

It has been reported previously that six pRNA molecules bind to the motor as a hexamer (Guo et al., 1998; Hendrix, 1998; Trottier and Guo, 1997; Zhang et al., 1998). As already noted, it is not necessary to add fresh pRNA to convert the intermediates into infectious virions (Fig. 3A). To test whether the procapsid-bound pRNA was needed during DNA translocation, RNase was added to cleave the motor-bound pRNA. It was found that after RNase treatment, the DNA-packaging intermediates could not be converted into infectious virions, while RNase did not affect the conversion of the finished DNA-filled capsid into an infectious virion (Fig. 3C). This shows that continued function of pRNA is needed for DNA translocation.

Discussion

Careful scrutiny of dsDNA viruses reveals a common feature that their DNA packaging motors involve two nonstructural components with at least one of them having characteristics of typical ATPases. A comparison among the nonstructural DNA-packaging proteins of the well-studied

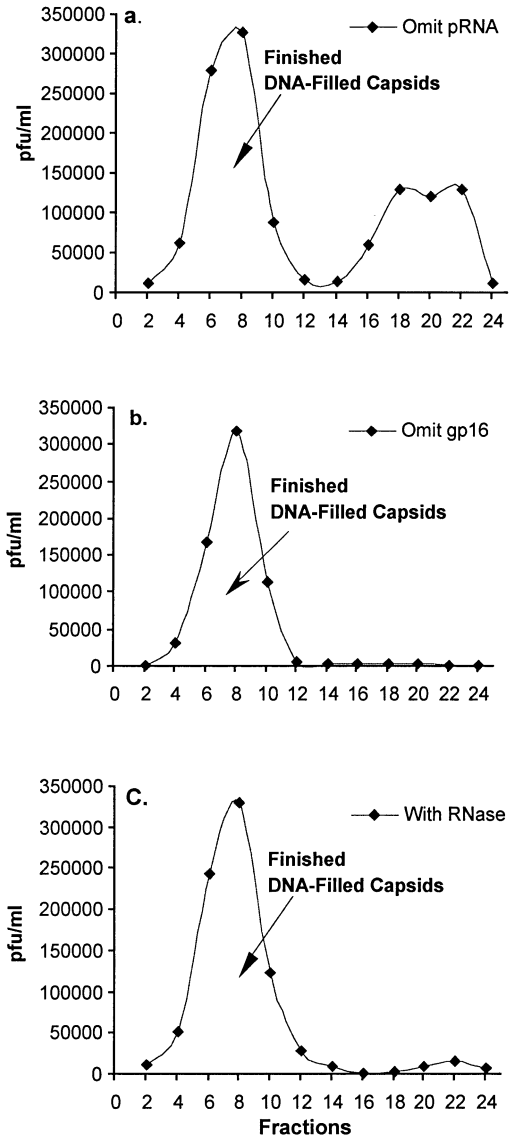

Fig. 3. Requirement of fresh gp16 and ATP but no requirement of pRNA for the completion of DNA packaging in intermediates. Each fraction of the gradient containing DNA-packaging intermediates from the DNA packaging reaction as subsequently converted into infectious phi29 virion with maturation components in the absence of pRNA (A), gp16 (B), or in the presence of RNase to cleave the pRNA in the intermediates (C).

phages separated the proteins into two groups (Guo et al., 1987). Group one is a procapsid binding, magnesium-binding, and DNA-dependent ATPase. Group two is a DNA-binding protein. It is interesting to find that the size of group one proteins is larger than that of group two proteins (Table 2 in Guo et al., 1987). Subsequent studies with other phages seem to support this distinction. For example, it has been reported that gpNu1 of lambda binds DNA and that the C-terminus of the DNA-dependent ATPase gp2 of phage 21 and gpA of lambda bind procapsid (Yeo and Feiss, 1995). The size of gpNuI is smaller than gpA. The data presented in this article suggest that pRNA is the counterpart of a group one DNA-packaging protein; that is, pRNA is the procapsid-binding component. pRNA might also be part of the ATPase complex as we have demonstrated that pRNA binds ATP (unpublished results). Overall these observations imply that pRNA is comparable to the members in group one, including gp17 of T_4 (Manne et al., 1982), gpA of lambda (Becker et al., 1977; Catalano et al., 1995), gp19 of T_3 and T_7 (Dunn and Studier, 1983; Yamada et al., 1986), gp2 of phage 21 (Yeo and Feiss, 1995), gp2 of P22 (Prevelige et al., 1990), gB of P1 (Skorupski et al., 1992), the terminase of psiM2 (Pfister et al., 1998) or Sfi21 (Desiere et al., 1998), gp1Y of phiC31 (Redenbach et al., 1996), UL15 of herpesvirus (Yu and Weller, 1998), IVA 2 of adenovirus (Bett et al., 1993; Schmid and Hearing, 1997; Zhang et al., 2001), and A32 of poxvirus (Koonin et al., 1993). However, gp16 also contains a consensus sequence for ATP binding. The role of gp16 in phi29 DNA packaging is still unclear. The binding of gp16 to procapsid has not been definitively demonstrated; one difficulty is the hydrophobic nature of gp16. The protein is insoluble after expression and self-aggregates after purification (Guo et al., 1986; Ibarra et al., 2001). The result in this article might provide insight on the function of gp16.

At least one component of a motor should act processively to keep it running constantly. Most biological motors, including myosin, kinesin, DNA-helicase, and RNA polymerase, involve one factor that acts processively. In phi29, DNA packaging involves the connector, pRNA, gp16, and ATP. The connector cannot be the processivity factor as the crystal structure reveals no potential ATP-binding pocket (Guasch et al., 2002; Simpson et al., 2000). Our results show that gp16 and pRNA are not needed to convert finished DNA-filled capsids into infectious viruses (Figs. 1, 2, and 3). This is understandable as DNA-packaging in these particles has been completed. The results also show that to convert the partially filled DNA-packaging intermediates into completed DNA-filled particles, fresh gp16 and ATP, but not pRNA, were needed (Fig. 3). This indicates that gp16 does not act processively during DNA translocation. However, the six copies of pRNA that are already bound to the motor are sufficient to complete DNA packaging. In addition, pRNA must be actively functioning during DNA packaging; when intermediates are treated with RNase, DNA packaging intermediates could not be matured into infectious virus (Fig. 3C). These data therefore suggest that only one pRNA hexamer is needed to package one copy of phi29 genomic DNA and that it functions continuously and processively during DNA packaging. Furthermore, the data also suggest that gp16 does not remain bound to the packaging complex as isolated packaging intermediates require the addition of gp16 to complete DNA encapsidation.

Materials and methods

Preparation of pRNA

RNAs were prepared as described previously (Zhang et al., 1994). Briefly, DNA oligos were synthesized with the desired sequences and used to produce double-stranded DNA by PCR. The DNA products containing the T_7 promoter were cloned into plasmids. RNA was synthesized with T_7 RNA polymerase by run-off transcription and purified from a polyacrylamide gel. The sequences of both plasmids and PCR products were confirmed by DNA sequencing.

In vitro phi29 DNA packaging

The purification of procapsids (Lee and Guo, 1995), gp16 (Guo et al., 1986) and DNA-gp3 (Guo et al., 1986; Ortin et al., 1971), the preparation of the tail protein (gp9), neck proteins (gp11, gp12), the morphogenetic factor (gp13) (Lee and Guo, 1995), and the procedure for DNA packaging in vitro have been described previously (Guo et al., 1986). Briefly, a quantity of 10 μl of purified procapsids was mixed with 1 μ1 pRNA (1 μg/μl) and dialyzed on a 0.025-μm membrane type VS filter (Millipore Corp.) against TBE (89 mM Tris–borate, pH 8.3, 2.5 mM EDTA) for 15 min at ambient temperature and then dialyzed against TMS (50 mM Tris–HCl, pH 7.8, 10 mM $MgCl_2$, 100 mM NaCl) for 30 min at ambient temperature. The presence of Mg^{2+} in TMS buffer promotes the binding of pRNA to the procapsid. These pRNA-enriched procapsids were mixed with 3 μ1 reaction buffer (10 mM ATP, 6 mM spermidine, 3 mM β-mercaptoethanol in TMS), 5 μ1 DNA-gp3 that had been dialyzed against TMS for 40 min at ambient temperature, and 6 μl DNA-gp3 packaging protein gp16 that had been dialyzed against gp16 buffer (0.01 M Tris–Cl, pH 7.5, and 0.04 M KCl) for 40 min on ice. These mixtures were then incubated for 30 min at ambient temperature. DNA packaging efficiency was verified by 5–20% sucrose gradient sedimentation (Guo et al., 1986) or agarose gel electrophoresis (Guo et al., 1991).

Maturation of phi29 DNA-packaging intermediates

DNA-packaging intermediates were generated by the addition of 5% of the poorly hydrolyzable ATP analogue

γ-S-ATP into the phi29 DNA packaging mixture. The intermediates were separated from free DNA and the finished DNA-filled procapsids by 5–20% sucrose gradient sedimentation with SW55 rotor for 35 min at 35,000 rpm. The gradients were fractionated to separate the components that have different sedimentation rates.

The components in each fraction of the gradient were subsequently converted into infectious phi29 virions by the addition of fresh components for phi29 in vitro assembly. The complete conversion system includes pRNA, gp16, ATP, neck protein gp11/12, and tail protein gp9. The converted products of infectious virions were titrated by plating on the bacterial host *Bacillus subtilis* Su^{+44}.

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References

Becker, A., Marko, M., Gold, M., 1977. Early events in the in vitro packaging of bacteriophage lambda DNA. Virology 78, 291–305.

Bett, A., Prevec, L., Graham, F., 1993. Packaging capacity and stability of human adenovirus type 5 vectors. J. Virol. 67, 5911–5921.

Black, L.W., 1989. DNA packaging in dsDNA bacteriophages. Ann. Rev. Microbiol. 43, 267–292.

Cassetti, MC., Merchlinsky, M., Wolffe, EJ., Weiserg, AS., Moss, B., 1998. DNA package mutant: repression of the vaccinia virus A32 gene results in noninfectious, DNA-deficient, spherical, enveloped particles. J. Virol. 72 (7), 5769–5780.

Catalano, C.E., Cue, D., Feiss, M., 1995. Virus DNA packaging: the strategy used by phage λ. Mol. Microbiol. 16, 1075–1086.

Desiere, F., Lucchini, S., Brussow, H., 1998. Evolution of Streptococcus thermophilus bacteriophage genomes by modular exchanges followed by point mutations and small deletions and insertions. Virology 241, 345–356.

Dunn, J.J., Studier, F.W., 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. 166, 477–535.

Earnshaw, W.C., Casjens, S.R., 1980. DNA packaging by the double-stranded DNA bacteriophages. Cell 21, 319–331.

Guasch, A., Pous, J., Ibarra, B., Gomis-Ruth, F.X., Valpuesta, J.M., Sousa, N., Carrascosa, J.L., Coll, M., 2002. Detailed architecture of a DNA translocating machine: the high-resolution structure of the bacteriophage phi29 connector particle. J. Mol. Biol. 315, 663–676.

Guo, P., 1994. Introduction: principles, perspectives, and potential applications in viral assembly. Sem. Virol. (Ed. Intro.) 5 (1), 1–3.

Guo, P., 2002. Structure and function of phi29 hexameric RNA that drive viral DNA packaging motor: review. Prog. Nucl Acid Res Mol. Biol. 72, 415–472.

Guo, P., Grimes, S., Anderson, D., 1986. A defined system for in vitro packaging of DNA-gp3 of the *Bacillus subtilis* bacteriophage φ29. Proc. Natl. Acad. Sci. USA 83, 3505–3509.

Guo, P., Peterson, C., Anderson, D., 1987. Prohead and DNA-gp3-dependent ATPase activity of the DNA packaging protein gp16 of bacteriophage φ29. J. Mol. Biol. 197, 229–236.

Guo, P., Rajogopal, B., Anderson, D., Erickson, S., Lee, C.-S., 1991. sRNA of bacteriophage φ29 of *B.subtilis* mediates DNA packaging of φ29 proheads assembled in *E. coli*. Virology 185, 395–400.

Guo, P., Zhang, C., Chen, C., Trottier, M., Garver, K., 1998. Inter-RNA interaction of phage phi29 pRNA to form a hexameric complex for viral DNA transportation. Mol. Cell. 2, 149–155.

Hackney, D.D., 1995. Highly processive microtubule-stimulated ATP hydrolysis by dimeric kinesin head domains. Nature 377, 448–450.

Harada, Y., Ohara, O., Takatsuki, A., Itoh, H., Shimamoto, N., Kinosita Jr., K., 2001. Direct observation of DNA rotation during transcription by *Escherichia coli* RNA polymerase. Nature 409, 113–115.

Hendrix, R.W., 1998. Bacteriophage DNA packaging: RNA gears in a DNA transport machine (Minireview). Cell 94, 147–150.

Hoeprich, S., Guo, P., 2002. Computer modeling of three-dimensional structure of DNA-packaging RNA(pRNA) monomer, dimer, and hexamer of Phi29 DNA packaging motor. J Biol. Chem. 277 (23), 20794–20803.

Ibarra, B., Valpuesta, J.M., Carrascosa, J.L., 2001. Purification and functional characterization of p16, the ATPase of the bacteriophage phi29 packaging machinary. Nucleic Acids Res. 29 (21), 4264–4273.

Inoue, A., Saito, J., Ikebe, R., Ikebe, M., 2002. Myosin IXb is a single-headed minus-end-directed processive motor. Nat. Cell Biol. 4, 302–306.

Jeruzalmi, D., O'Donnell, M., Kuriyan, J., 2001. Crystal structure of the processivity clamp loader gamma (gamma) complex of *E. coli* DNA polymerase III. Cell 106, 429–441.

Koonin, E.V., Senkevich, V.I., Chernos, V.I., 1993. Gene A32 product of vaccinia virus may be an ATPase involved in viral DNA packaging as indicated by sequence comparisons with other putative viral ATPases. Virus Genes 7, 89–94.

Lee, C.S., Guo, P., 1995. In vitro assembly of infectious virions of ds-DNA phage φ29 from cloned gene products and synthetic nucleic acids. J. Virol. 69, 5018–5023.

Manne, V.S., Rao, V.B., Black, L.W., 1982. A bacteriophage T4 DNA packaging related DNA-dependent ATPase-endonuclease. J Biol Chem 257, 13223–13232.

Molineux, I.J., 2001. No syringes please, ejection of phage T7 DNA from the virion is enzyme driven. Mol. Microbiol. 40, 1–8.

Morita, M., Tasaka, M., Fujisawa, H., 1993. DNA packaging ATPase of bacteriophage T3. Virology 193, 748–752.

Ortin, J., Viñuela, E., Salas, M., Vásquez, C., 1971. DNA-protein complex in circular DNA from phage φ29. Na. New Biol. 234, 275–277.

Pang, P.S., Jankowsky, E., Planet, P.J., Pyle, A.M., 2002. The hepatitis C viral NS3 protein is a processive DNA helicase with cofactor enhanced RNA unwinding. EMBO J. 21, 1168–1176.

Pfister, P., Wasserfallen, A., Stettler, R., Leisinger, T., 1998. Molecular analysis of methanobacterium phage psiM2. Mol. Microbiol. 30, 233–244.

Prevelige, P.E.J., Thomas, D., King, J., Towse, S.A., Thomas, G.J.J., 1990. Conformational states of the bacteriophage P22 capsid subunit in relation to self-assembly. Biochemistry 29, 5626–5633.

Redenbach, M., Kieser, H.M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., Hopwood, D.A., 1996. A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. Mol. Microbiol. 21, 77–96.

Romberg, L., Vale, R.D., 1993. Chemomechanical cycle of kinesin differs from that of myosin. Nature 361, 168–170.

Schmid, S.I., Hearing, P., 1997. Bipartite structure and functional independence of adenovirus type 5 packaging elements. J Virol. 71, 3375–3384.

Schnitzer, M.J., Block, S.M., 1997. Kinesin hydrolyses one ATP per 8-nm step. Nature 388, 386–390.

Simpson, A.A., Tao, Y., Leiman, P.G., Badasso, M.O., He, Y., Jardine, P.J., Olson, N.H., Morais, M.C., Grimes, S., Anderson, D.L., Baker, T.S., Rossmann, M.G., 2000. Structure of the bacteriophage phi29 DNA packaging motor. Nature 408, 745–750.

Skorupski, K., Pierce, J.C., Sauer, B., Sternberg, N., 1992. Bacteriophage P1 genes involved in the recognition and cleavage of the phage packaging site (pac). J. Mol. Biol. 223, 977–989.

Smith, D.E., Tans, S.J., Smith, S.B., Grimes, S., Anderson, D.L., Bustamante, C., 2001. The bacteriophage phi29 portal motor can package DNA against a large internal force. Nature 413, 748–752.

Trottier, M., Guo, P., 1997. Approaches to determine stoichiometry of viral assembly components. J. Virol. 71, 487–494.

West, S.C., 1996. DNA helicases: new breeds of translocating motors and molecular pumps. Cell 86, 177–180.

Wirtz, E., Hoek, M., Cross, G.A., 1998. Regulated processive transcription of chromatin by T7 RNA polymerase in *Trypanosoma brucei*. Nucleic Acids Res. 26, 4626–4634.

Wunderli, H., vd, B.J., Kellenberger, E., 1977. Studies related to the head-maturation pathway of bacteriophages T4 and T2. I. morphology and kinetics of intracellular particles produced by mutants in the maturation genes. J Supramol. Struct. 7, 135–161.

Yamada, M., Fujisawa, H., Kato, H., Hamada, K., Minagawa, T., 1986. Cloning and sequencing of the genetic right end of bacteriophage T3 DNA. Virology 151, 350–361.

Yeo, A., Feiss, M., 1995. Specific interaction of terminase, the DNA packaging enzyme of bacteriophage lambda, with the portal protein of the prohead. J.Mol.Biol. 245, 141–150.

Yu, D., Weller, S.K., 1998. Generic analysis of the UL15 gene locus for the putative terminase of herpes simplex virus type 1. Virology 243, 32–44.

Zhang, C.L., Lee, C.-S., Guo, P., 1994. The proximate 5′ and 3′ ends of the 120-base viral RNA (pRNA) are crucial for the packaging of bacteriophage ϕ29 DNA. Virology 201, 77–85.

Zhang, F., Lemieux, S., Wu, X., St.-Arnaud, S., McMurray, C.T., Major, F., Anderson, D., 1998. Function of hexameric RNA in packaging of bacteriophage phi29 DNA in vitro. Mol. Cell. 2, 141–147.

Zhang, W., Low, J.A., Christensen, J.B., Imperiale, M.J., 2001. Role for the adenovirus IVa2 protein in packaging of viral DNA. J. Virol. 75, 10446–10454.