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The ATPase Domain of the Large Terminase Protein, gp17, from Bacteriophage T4 Binds DNA: Implications to the DNA Packaging Mechanism

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Translocation of double-stranded DNA into a preformed capsid by tailed bacteriophages is driven by powerful motors assembled at the special portal vertex. The motor is thought to drive processive cycles of DNA binding, movement, and release to package the viral genome. In phage T4, there is evidence that the large terminase protein, gene product 17 (gp17), assembles into a multisubunit motor and translocates DNA by an inchworm mechanism. gp17 consists of two domains; an N-terminal ATPase domain (amino acids 1–360) that powers translocation of DNA, and a C-terminal nuclease domain (amino acids 361–610) that cuts concatemeric DNA to generate a headful-size viral genome. While the functional motifs of ATPase and nuclease have been well defined and the ATPase atomic structure has been solved, the DNA binding motif(s) responsible for viral DNA recognition, cutting, and translocation are unknown. Here we report the first evidence for the presence of a double-stranded DNA binding activity in the gp17 ATPase domain. Binding to DNA is sensitive to Mg^{2+} and salt, but not the type of DNA used. DNA fragments as short as 20 bp can bind to the ATPase but preferential binding was observed to DNA greater than 1 kb. A high molecular weight ATPase–DNA complex was isolated by gel filtration, suggesting oligomerization of ATPase following DNA interaction. DNA binding was not observed with the full-length gp17, or the C-terminal nuclease domain. The small terminase protein, gp16, inhibited DNA binding, which was further accentuated by ATP. The presence of a DNA binding site in the ATPase domain and its binding properties implicate a role in the DNA packaging mechanism.

Keywords: virus assembly; bacteriophage T4; DNA packaging; ATPase; DNA binding

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Introduction

Viral genome packaging transforms a highly metabolically active intracellular DNA into one that is tightly packaged to near-crystalline density within the confines of a protein shell.[1] In most tailed bacteriophages, a multisubunit terminase complex recognizes the newly replicated concatemeric DNA, makes an endonucleolytic cut, and links it to the dodecameric portal ring located at a special vertex of the icosahedral capsid.[2] The packaging machine transports DNA through the portal channel, powered by ATP hydrolysis energy.[3] In phage ϕ29, an average of 2 bp is translocated per one ATP hydrolysis and a force as high as 80 pN is generated.[4,5] The phage T4 motor, packaging a 171-kb genome at a rate of up to ~2000 bp/s, is the fastest and the most powerful motor reported to date.[6] After packaging one headful length DNA, which is equivalent to one (e.g., phage λ) or slightly more than one (e.g., phage T4) genome length, a second endonucleolytic cleavage disconnects the terminase–DNA complex from the packed capsid, allowing assembly of neck and tail components to complete infectious virion assembly.[7]

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Abbreviations used: dsDNA, double-stranded DNA; GF, gel filtration; gp, gene product(s); HTH, helix–turn–helix; ssDNA, single-stranded DNA.

Terminase is a key component of this highly dynamic packaging process. The phage T4 terminase is composed of the small terminase protein, gene product 16 (gp16) (18 kDa), and the large terminase protein, gp17 (70 kDa).[8,9] gp16 exists as an oligomer in solution, apparently as an octamer, whereas the gp17 exists as a monomer.[9,10] Although the stoichiometry is unknown, a holoterminase complex formed by the interaction of gp16 and gp17 is responsible for carrying out the key steps involved in DNA packaging: viral DNA recognition, DNA cleavage for packaging initiation and termination, and DNA translocation.[11] The gp17 is the catalytic component, possessing ATPase, nuclease, and *in vitro* DNA packaging activities, whereas gp16 is a regulator, stimulating the gp17-associated ATPase and packaging activities by 50- to 100-fold.[9,12–14] gp17 alone is capable of assembling on the prohead as a functional motor, efficiently packaging any externally added linear double-stranded DNA (dsDNA) in a defined system.[6,15–17]

Three critical functional motifs of the phage terminase complex have been well characterized (Fig. 1a): (i) The initial recognition of viral DNA is mediated by a helix–turn–helix (HTH) motif present in the small terminase protein, an activity that is best characterized in the phage λ small terminase, gpNu1.[1] (ii) The DNA cuts for packaging initiation and termination are evidently carried out by a catalytic metal-binding cluster present in the C-terminal nuclease domain of gp17 and other large terminase proteins.[18] (iii) The DNA translocation is powered by an ATPase catalytic center present in the N-terminal domain of large terminase proteins.[12,15] In the only atomic structure solved, the T4 packaging ATPase features a RecA-like αβ-core domain, exhibiting closest similarity to monomeric SF1 and SF2 helicases.[19] The functional signatures of the ATPase such as Walker A, Walker B, catalytic carboxylate, and ATPase coupling motifs have been well defined through molecular genetic and biochemical studies. Conservative substitutions in any of the motifs result in a complete loss of ATPase and DNA packaging activities, suggesting a tight coupling between the two functions.[20–23]

The identity of DNA binding motifs, which are also central to genome packaging, however, remained unknown. At minimum, three key steps

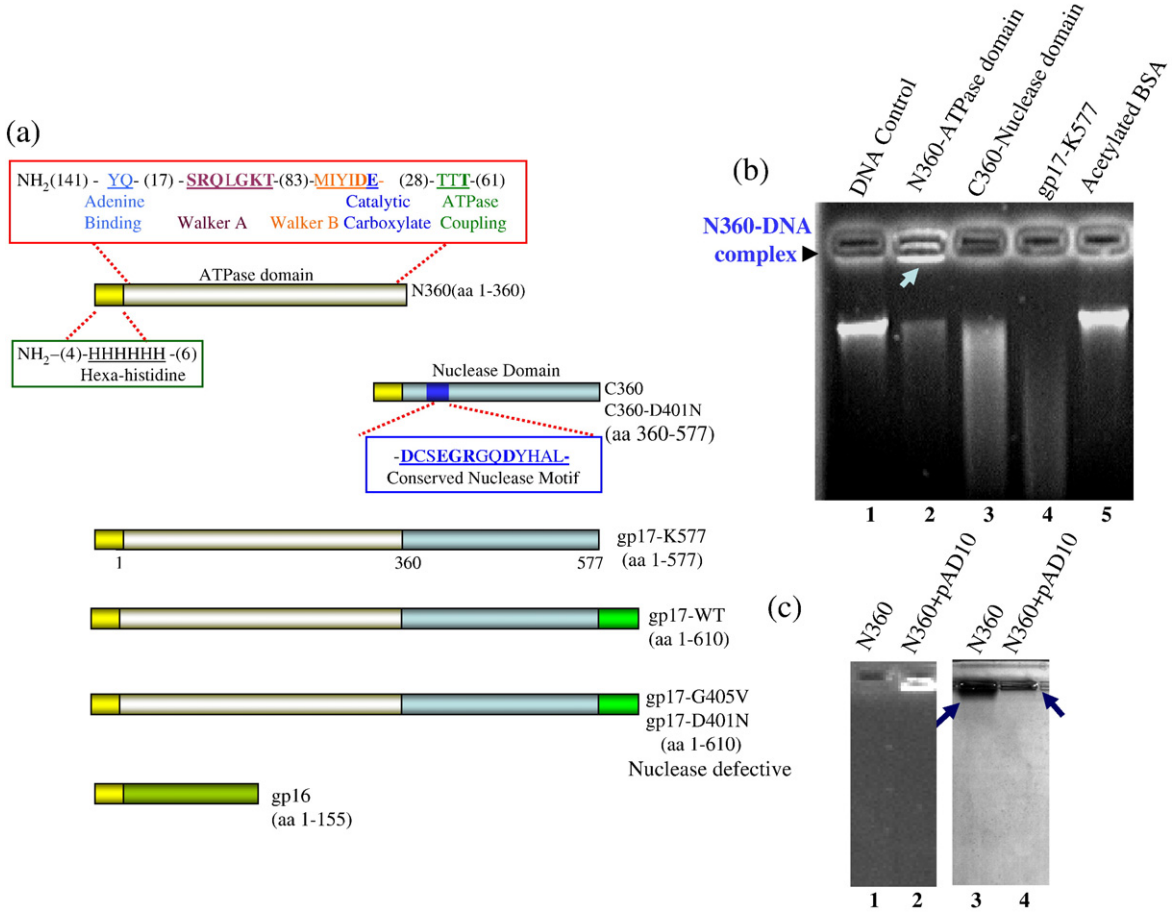

Fig. 1. The gp17–ATPase domain binds DNA. (a) Schematic of the terminase protein constructs used in the study. The basic motifs are shown in the rectangular boxes; numbers represent amino acids; the amino acids shown in bold are shown to be critical for function. The functional characterization of the motifs and mutants were described previously.[18,20–23] (b) Nuclease activity of gp17 and its domains as per the procedure described in Materials and Methods. (c) Agarose gel showing trapping of N360 or N360–DNA complex in the well. The gel was stained with either ethidium bromide (left) or Coomassie blue-G250 (right). The arrows indicate the trapped N360 or N360–DNA complex.