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DNA Packaging: A New Class of Molecular Motors

Dispatch

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DNA is packaged into preformed bacteriophage capsids to liquid crystalline density by the action of a portal protein complex. Single molecule packaging studies indicate that this is a new and extremely powerful class of molecular motors.

Viral genomes are contained within, and protected by, proteinaceous shells termed capsids. Typically, the capsid is composed of anywhere from approximately 200 to 2000 identical or structurally related protein subunits. In addition to the protein subunits that make up the protective shell, the proteins required to support the virus lifecycle — such as receptor binding proteins, fusion proteins and polymerases — are also specifically incorporated during capsid assembly [1]. Despite the striking success of X-ray crystallography and electron cryomicroscopy in determining their structure, virus capsids are not static containers that function solely to protect the nucleic acid from environmental insult. Rather, emerging structural and biochemical evidence suggests that capsids are dynamic specialized macromolecular machines. This picture is reinforced by a new biophysical study which has directly measured the forces involved in packaging DNA into a bacteriophage capsid [2].

As an example of the dynamic properties of virus capsids, the binding of polio virus to its cellular receptor, Pvr, triggers a global conformational change within the capsid that results in loss of the internal viral protein VP4 and displacement of the amino terminus of the VP1 capsid protein from the interior to the viral surface [3]. This structural change facilitates viral fusion and entry. In the case of reovirus, a double-stranded RNA-containing virus, a protective inner core can be found inside infected cells. Pentameric enzyme complexes composed of protein λ2 are located at each of the twelve icosahedral vertices of the reovirus core particle, and act both to add a methylated guanosine cap to the 5' end of the viral mRNA and to extrude the mRNA into the cytoplasm [4].

As might be expected, building complex macromolecular machines is not a simple business, and the assembly pathways of many viruses reflect this complexity. While some capsids appear to form by simple co-condensation of the viral nucleic acid with the capsid protein, others, such as the capsids of double-stranded DNA-containing bacteriophages and herpes viruses, first assemble an empty procapsid into which the DNA is packaged (Figure 1) [5].

Procapsids are intricate structures, the assembly of which requires the transient presence of several hundred molecules of scaffolding protein which acts both to catalyze and chaperone the assembly process. The scaffolding protein can be found inside the procapsid. Positioned at one of the twelve icosahedral vertices is a dodecameric complex of the virus-encoded portal protein. This dodecameric complex, known as the portal or connector complex, forms the channel through which the viral DNA is packaged into the capsid, and through which it exits during infection. While the portal proteins from different phage show relatively little sequence homology and vary widely in molecular weight, portal complexes display significant morphological similarity as determined by electron microscopy [6]. Morphologically, they present as disk-like structures approximately 150 Å in diameter with radially arranged projections and a 30 Å central channel.

The portal dodecamer is located at, and probably replaces, a five-fold vertex of the viral capsid, and thus represents a symmetry mismatch [6–8]. Because there can be no simple one-to-one correspondence of binding interactions between the capsid lattice and the portal complex, the symmetry mismatch has intriguing functional and morphogenic consequences. It has been suggested that, by preventing all bonding interactions from being either completely in or out of phase simultaneously, the symmetry mismatch allows for smooth rotation of the portal protein during DNA packaging [9].

The DNA packaging reaction results in the removal of the scaffolding protein from the procapsid, either by proteolysis or extrusion, and triggers a conformational rearrangement of the proteins within the capsid lattice that renders the structure more stable [10]. Interestingly, procapsids are assembled as metastable intermediates, and the structural transformations that accompany DNA packaging are energetically downhill, so the procapsid is like a spring-loaded protein shell [11]. The DNA within the capsid, while remaining in B-form, is packaged to liquid crystalline-like density, and considerable effort has been expended in attempts to decipher the arrangement of the condensed DNA within the capsid [12]. While a wide variety of models — including the coaxial spool, spiral fold, liquid crystal and folded toroid — have been proposed, current evidence favors a model in which the strands of the DNA are wound around a common axis in approximately six coaxial spools [13,14].

The packaging reaction is energy dependent and typically involves several components. ATP hydrolysis provides the driving force, and it is estimated that one ATP molecule is required for every base pair that is

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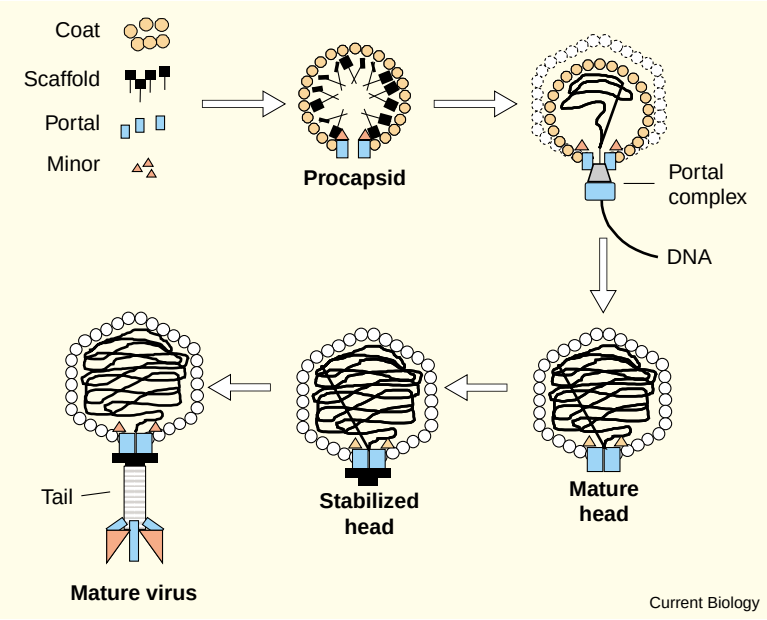

Figure 1. The morphogenesis of a tailed double-stranded DNA bacteriophage.

Scaffolding proteins direct the structural proteins of the phage head to polymerize into a procapsid with icosahedral symmetry. Located at one vertex in the procapsid is a portal complex, which is recognized by a terminase complex and pumps DNA into the head in an ATP-dependent reaction. Packaging induces a structural change in the capsid lattice, leading to increased stability. Following DNA packaging, the DNA is cleaved and the mature head is stabilized by the addition of stabilization proteins. Pre-assembled tails then add to render the virus infectious.

packaged [15]. The ATPase activity is harbored in one subunit of two protein molecules comprising the 'terminase' complex. In addition to providing the ATPase activity, the terminase recognizes a specific DNA sequence to ensure selective packaging of the virus genome. The terminase complex binds to the portal protein complex delivering the DNA to the packaging site.

The structure of one phage portal protein, that of the ϕ29 connector, has been determined at atomic resolution [16]. The connector itself is comprised of a 12-subunit ring containing extensive α-helical secondary structure, with three long helices of each subunit forming the central channel. The ring is 138 Å across at the wide end and narrows to 66 Å at bottom. The internal channel is 60 Å at the top and 36 Å at the bottom. The channel is lined with negatively charged amino acids at the wide end which may repel DNA during translocation. The smooth external surface of the stem region has no significant charge density, suggesting that rotation (if it occurs) may be facilitated by a non-specific, oily interface between the connector and prohead.

The connector sits in the prohead with the large portion of the ring on the inside and the narrow end partially extending out of the capsid [16]. A clear channel can be seen through the connector in micrographs indicating that there is no capsid density at the portal vertex. The assembled connector is too large to fit through the opening in the capsid at the portal vertex, suggesting that the portal ring is probably incorporated before the prohead is completely assembled. A unique feature of the ϕ29 connector is the presence of a 174 base pRNA, which is required for packaging [17]. While biochemical studies have suggested that this pRNA forms a hexameric ring in solution [18], structural studies suggest that it forms a pentameric ring encircling the protruding end of the portal protein and contacting the capsid lattice [16]. Flash-frozen samples of actively packing proheads provide structural evidence for the hypothesis that the DNA translocates through the central channel [16].

A number of models of how packaging may be accomplished have been proposed [9,16,19]. In one model that is consistent with the biochemical and structure data, one subunit of the portal dodecamer interacts with the DNA loaded in the central channel. ATP hydrolysis then drives a 12° rotation of the narrow end of the connector, resulting in a lengthwise expansion of the connector via a slight change in the angle of the long helices. In a subsequent step, the wide end of the connector follows the narrow end, allowing the structure to relax and contract while translating two base pairs of DNA into the capsid [16].

The high efficiency of the ϕ29 *in vitro* packaging system has recently made it possible to measure directly the forces involved in DNA packaging [2]. In these experiments, the biotinylated DNA tail protruding from stalled, partially prepackaged complexes was attached to a polystyrene bead captured in an optical trap, and the capsid was captured by tethering to another bead coated with anti-capsid antibodies. Upon the addition of ATP, the two beads moved closer together indicating packaging is occurring. Detailed analysis of the packaging profiles revealed that the motors are highly efficient: 95% of the complexes studied displayed movements of several micrometres. Packaging the entire 6.6 μm, 19 kilobase long genome was found to require about five minutes, and during packaging approximately eighteen four-second pauses were observed. During these pauses, the DNA did not slip, indicating that the motor remained engaged; but there were occasional slippages averaging 44 base pairs in length, after which

the motor did immediately reengage and continue to package.

The packaging rate was initially ~100 base pairs per second but dropped to zero as the capsid filled up and the motor stalled. A marked transition in packaging rate was found to occur when the capsid is approximately 50% full. These data suggest that the rate decreases because of a build up of pressure within the capsid. Stall force measurements suggest that the motor can drive DNA into the head with an approximate 30% efficiency until the internal force builds to ~50 pN. This makes the portal complex one of the strongest molecular motors studied to date, with a strength approximately eight times that of kinesin and twice that of RNA polymerase.

On the basis of this force, and the cross-sectional area of the packaged DNA, a pressure of 6 MPa within the capsid can be calculated, and the tensile strength necessary to contain this force is comparable to that of typical aluminum alloys. It is possible that this force is available to assist in injection of the phage DNA into the host cell to facilitate infection [2].

It appears that the portal motor may represent a new and extremely powerful class of motor which couples rotation to DNA translocation. Some fascinating questions remain to be answered. Does the portal in fact rotate? Do conformational changes in the subunits occur, and are they coupled or do the subunits undergo individual conformational changes? What is the detailed mechanism of the movement? What is the role of the pRNA in this movement? In those phage whose portal appears to lack a pRNA, is this function replaced by a domain of the protein? And how is such a complex and dynamic structure assembled with high fidelity during phage morphogenesis?

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