Structure of the T7 packaging motor: implications in DNA translocation

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Viral DNA packaging is an attractive system to study the main features of DNA-protein interactions due to the high number of functions and sequential interactions it entails. DNA packaging inside preformed proheads takes place in a similar way in Caudoviridae bacteriophages as well as in some animal viruses (such as Herpesvirus and Adenovirus). The structural and functional complexity of phages accomplished by its genetic simplicity makes them a good model system to study viral packaging. The morphogenetic pathway in double stranded DNA (dsDNA) phages, except for some individual features, is highly conserved owing to their common evolutionary origin. The assembly begins with the formation of empty proheads composed by the capsid proteins, the scaffolding proteins and the connector (that links the viral head and the tail). DNA is translocated inside the procapsid passing through the internal channel of the connector with ATP consumption. This process is performed by the connector and a complex called terminase, which recognizes DNA and presents ATPase and nuclease activity. The terminase has been proposed as the macromolecular motor that converts chemical energy from ATP hydrolysis into mechanical movement of DNA inside the prohead. It is formed by two subunits whose precise stoichiometry has not yet been directly demonstrated. Once the DNA is densely packaged inside the mature head, the terminase complex dissociates from the prohead. The viral assembly pathway is finished with the incorporation of the tail proteins to build the fully infective virion (reviewed in [1]).

Our studies focus on the structural and functional characterization of the terminase complex of phage T7. We have cloned the major subunit of the complex (gene product 19) which hydrolyzes ATP, cleaves the DNA and is essential for *in vitro* packaging assays [2]. We have purified the functional oligomer and the use of GraFix centrifugation [3] allowed us to start its structural characterization using electron microscopy (EM) and image processing techniques. As there was no direct evidence of its oligomeric structure, we performed a two dimensional image analysis, based on maximum-likelihood multi-reference alignment methods and rotational spectra, which clearly showed its 5 fold symmetry. The subsequent three dimensional analysis using XMIPP [4] and EMAN software [5] confirmed so. The symmetry of the terminase defines the matches and mismatches needed for DNA translocation. In addition, the precise mechanism of packaging still remains unclear as the catalytic regions within the terminase structure need to be located. To this end, using as a template the already solved atomic structure of a similar protein [6], we obtained an atomic model of the monomeric and pentameric T7 terminase. The accuracy of the fitting inside our EM volume at 17 Å resolution allowed us to localize the main functional regions and to map the corresponding electrostatic surfaces. The accessibility of the catalytic domains and the asymmetric distribution of the charges in the pentamer would, respectively, allow the interaction with the nucleotide and also the DNA translocation.

The functioning of the terminase complex is tightly related to its interaction with the connector, through which it binds to the prohead. Moreover, the symmetry mismatch between the dodecameric connector and the pentameric large terminase may condition their coupling during DNA translocation. Hence we are presently performing the structural analysis of the complex formed by the connector and the large terminase. This might clarify not only the interaction of the packaging motor with both the prohead and the DNA, but eventually the conformational changes required for DNA translocation.

References

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