

MATURATION AND QUASIEQUIVALENCE IN THE COVALENTLY LINKED CAPSID OF PHAGE HK97

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The mature empty capsid of phage HK97 is composed of 420 protein units in an icosahedral T=7 lattice. The subunits are covalently linked into five- and six-membered rings through a lysine-asparagine cross-link. These rings are topologically linked, connecting all 420 subunits into a single stable unit. The coat protein has a unique fold and consists of two domains and two extended regions responsible for a complicated pattern of interactions in the mature capsid. The protein shell undergoes large conformational changes during particle maturation. In the mature shell, there are only few differences in the quasi-equivalent five-fold and six-fold interactions, while the capsids initially are formed by skewed hexamers. We have now refined the crystal structure of the mature capsids to 3.44 Å resolution to analyze the interactions between the subunits and correlate this with the low-resolution structures of the intermediates in the maturation process.

Keywords: VIRUS ASSEMBLY BACTERIOPHAGE CROSS-LINK

STRUCTURE OF THE BACTERIOPHAGE PHI29 DNA PACKAGING MOTOR

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Motors generating mechanical force, powered by the hydrolysis of ATP, are used to translocate double-stranded DNA into preformed capsids (proheads) of bacterial viruses and certain animal viruses. Here, we describe the motor that packages the dsDNA of the *Bacillus subtilis* bacteriophage φ29 into a precursor capsid. The structure of the head-tail connector, the central component of the phi29 DNA packaging motor, was determined to 3.2 Å resolution by means of X-ray crystallography. The connector was fitted into the electron density of the prohead and the partially packaged prohead determined by cryo-electron microscopy image reconstructions. Our results suggest that the prohead plus dodecameric connector, prohead RNA (pRNA), viral ATPase, and DNA comprise a rotary motor with the head-pRNA-ATPase complex acting as a stator, the DNA acting as a spindle, and the connector as a ball-race. The helical nature of the DNA converts the rotary action of the connector into translation of the DNA.

Keywords: PHI29 PHAGE, DNA PACKAGING, CRYOEM

THE ATOMIC STRUCTURE OF A DOUBLE SHELLED VIRUS, RICE DWARF VIRUS

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Rice dwarf virus (rdv) is a member of reoviridae genus phyto-reovirus possessing a double-stranded rna genome of 12 segments enclosed within an icosahedral double-shelled particle. It infects rice, wheat, barley, and other gramineae through insect vectors, leafhoppers (main vector nephotettix species), causing chlorotic flecks at the site of infection and stunting of the plant bodies. The outer capsid consists of 780 subunits of 46 kda protein p8 and the inner capsid consists of 120 subunits of 114 kda protein p3. This double-shelled capsid packages the twelve double stranded rnas, p1, p5 and p7 proteins inside the viral core. The 164 kda protein p1 is an rna dependent rna polymerase and the 55 kda protein p7 is an rna binding protein. The 91kda protein p5 is shown to possess gtp-binding activity and is the putative viral guanylyltransferase. A further minor 123 kda capsid protein, p2, plays a role in virus infection of vector cells. The proteins p2 and p8 are associated with viral transmission by the insect vector. The rdv crystal contains all viral components except the protein p2, which is eliminated during the purification process. The 26-angstrom resolution cryo-electron microscope map was used as a starting model. Fifteen-fold non-crystallographic symmetry averaging phase extension was succeeded to extend the resolution to 3.5 Å, giving a good quality electron density map. We have succeeded to build an atomic model of the double-shelled region, which gives useful information on the structural organization of viral capsid components to build a whole virus particle.

Keywords: RICE DWARF VIRUS VIRUS STRUCTURE MACROMOLECULAR ASSEMBLIES

THE STRUCTURE OF A PARAMYXOVIRAL FUSION PROTEIN - A NOVEL PARADIGM FOR THE MECHANISM OF MEMBRANE FUSION

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The structure of the ectodomain of the fusion glycoprotein of Newcastle disease virus has been determined to 3.3 Å resolution via X-ray crystallography. The observed molecule is a homotrimer with a club-like shape, morphologically consistent with images, which we obtained via negative stain electron microscopy. The trimer can be divided into three structural regions which we have termed the head, neck and stalk regions. Within each monomer, the head is further sub-divided into an irregular beta-barrel domain and an immunoglobulin domain, these comprising polypeptide predominantly from F1. The neck consists of a primarily-parallel β-sheet and an α-helix, which together surround an irregular four-helical bundle and the C-terminal region of the so-called HR-A coiled coil. The stalk is then formed by the remainder of the HR-A coiled coil. Considerable portions of the F0 polypeptide appear disordered or absent in the crystal; these include the extreme N-terminal region of HR-A, the fusion peptide and all residues within and adjacent to HR-B. Large axial and radial channels are visible within the head and neck regions and we postulate that these may play a role in sequestering the fusion peptide. On the basis of the structure we propose possible models for the structural transitions in NDV-F that accompany the fusion event.

Keywords: FUSION PROTEIN NEWCASTLE DISEASE VIRUS PARAMYXOVIRUS