Friday, March 23, Session V

L17

NEAR-ATOMIC STRUCTURE OF PODOVIRUS P68 PROVIDES INSIGHTS INTO PHAGE ASSEMBLY AND CELL MEMBRANE PENETRATION MECHANISM OF BACTERIOPHAGES INFECTING GRAM-POSITIVE BACTERIA

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Bacteriophages from family *Podoviridae* are characteristic by their short tails. Bacteriophage P68 is dsDNA virus infecting *Staphylococcus aureus*. To penetrate in the host cell, the phage tail is equipped with proteins that cleave a pore in the bacterial cell wall and cell membrane. Subsequently, the phage genome is ejected into the host cell where the replication process takes place. However, there is limited information about the native structure and cell penetration mechanism of *Podoviridae* phages infecting gram-positive bacteria.

Here we present *in situ* cryo-EM structure of the full capsid and tail at resolutions of 3.3 Å and 3.9 Å respectively. The atomic model reveals unique interconnections between phage structural proteins, and structural differences between full and empty particle show proteins involved in membrane penetration. We found two novel proteins (Arstotzka protein and Acne protein) in capsid, which have not been observed before. Structural analysis of the Arstotzka protein suggests its participation in dsDNA and capsid stabilization, while structural and sequential analysis of the Acne protein has shown its involvement in receptor binding. Native structure of the tail revealed 72 copies of a unique structural protein forming dodecameric ring positioned between portal and dsDNA, which is not present in the electron density map of the empty particle. Sequence analysis and electron microscopy shows that this protein might be released before dsDNA and penetrate the bacterial cell membrane. Furthermore, we found out that the structure of receptor binding protein (RBP) from P68 solved at 2.0 Å resolution by X-ray crystallography is similar to RBP from family *Siphoviridae.*

Here we present a function of different structural proteins of P68, and show how native particle of bacteriophage P68 is assembled. These findings demonstrate that there is more than one mechanism of the cell membrane penetration in the family *Podoviridae*, and that the receptor binding mechanisms are conserved among different families of bacteriophages infecting gram-positive bacteria.

L18

CRYO-EM STRUCTURE OF KASHMIR BEE VIRUS

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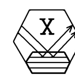
Kashmir bee virus (KBV) infects many species of insect pollinators, including agriculturally indispensable honeybees *Apis Mellifera* and *Apis Cerana.* KBV replicates faster and can be more lethal than other viruses of honeybees, such as sacbrood bee virus, deformed wing virus and black queen cell virus. However, KBV can also persist in seemingly healthy insects for a long time as an unapparent infection. Like the other members of family *Dicitroviridae*, non-enveloped KBV virions contain positive sense single stranded RNA genome, coated with capsid, which is composed of three major capsid proteins VP1, VP2, VP3, and minor protein VP4. KBV is part of the acute bee paralysis – Kashmir bee – Israeli bee paralysis virus cluster of related viruses that are together worldwide distributed. Despite of the overall genome sequence identity of IAPV and KBV of 76% and amino acids sequence identity of the capsid proteins of 75%, the viruses cause different symptoms and have distinct structural properties. Here we report the structure of KBV virion determined to the resolution of 2.9 Å using cryo-electron microscopy. We show, that VP4 protein of KBV has two -helixes at its C-terminus. These helices may increase the stability of the KBV capsid relative to the one of IAPV. We also show that VP3 protein forms a -strand on its N-terminus. Five of these strands form a beta-annulus around fivefold axis of the virus capsid. This feature has not been observed before in any virus from the family *Dicistroviridae*.



L19

MICROTUBULE ASSOCIATED PROTEIN 2c AND Tau: INSIGHT INTO MOLECULAR BASIS OF FUNCTIONAL DIVERSITY OF HOMOLOGOUS REGULATORY INTRINSICALLY DISORDERED PROTEINS

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Microtubule Associated Proteins (MAP) play a key role in regulation of microtubule (MT) dynamics. Products of two genes, *MAPT* and *MAP2*, are expressed in nerve cells in multiple isoforms generated by alternative splicing. The former gene encodes protein Tau, which is associated with neurodegenerative processes accompanying the Alzheimer's disease, and has therefore attracted great attention in the past few decades. Microtubule associated protein 2c (MAP2c), the shortest, 467-residue, 49 kDa isoform of the latter gene, is involved in neuronal development and is less characterized than its homologue Tau.

MAP2c consists of several structural and functional regions. The N-terminal domain contains two important parts: The N-terminal region with a high content of negatively charged amino acids and the proline-rich region. The former segment contains a proposed binding site for steroids, while the latter one interacts with SH3 domain of plectin [1] which act as a cytolinker and regulates actin dynamics. The second important part of MAP2c is a highly-conserved C-terminal domain that binds to MTs. Our goal was to characterize the MAP2c interactions, make a comparison to Tau, and deduce the functional differences between MAP2c and Tau.

Identification of structural motifs responsible for specific functions of MAP2c and Tau is complicated by the fact that MAP2c and Tau belong to the class of intrinsically disordered proteins (IDPs) lacking a unique structure and exist in multiple, quickly interconverting conformations. Given the disordered nature of IDPs, NMR is a key experimental method for studying IDPs. We used high-resolution NMR techniques and small angle X-ray scattering to acquire atomic-resolution data reflecting structural and dynamic features of MAP2c [2]. The results allowed us to correlate structural features and dynamics of MAP2c with its known and proposed binding and phosphorylation sites, and to directly compare MAP2c with Tau.

We obtained a detailed description of the transient secondary and tertiary structure of MAP2c. We also identified intramolecular contacts, caused by electrostatic interactions, with a great impact on dynamics of MAP2c. And most importantly, we revealed differences in phosphorylation of Tau and MAP2c, with important consequences for interactions of these proteins.

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L20

VIRION STRUCTURES AND GENOME RELEASE MECHANISM OF HONEYBEE VIRUSES FROM THE FAMILY *IFLAVIRIDAE*

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The worldwide population of western honeybee (*Apis mellifera*), the most economically important pollinator, is under pressure from habitat loss, environmental stress and diseases. Viruses from the family *Iflaviridae* cause lethal diseases in honeybees and other wild pollinators such as bumblebees (*Bombus sp.*). Two most important honeybee viruses from this family are deformed wing virus (DWV) and slow bee paralysis virus (SBPV).

Here we present the virion structures of DWV and SBPV determined by cryo-electron microscopy and X-ray crystallography. Capsid proteins VP3 of both of the viruses have C-terminal extension that fold into globular protruding (P)-domains exposed at the virion surfaces. Similar domains have not been previously observed in other viruses from the order Picornavirales. The P-domain contains conserved Asp-His-Ser catalytic triad. These residues may participate in receptor binding or provide the protease, lipase, or esterase activity required for the entry of the virus into the host cell. Furthermore, nucleotides from the DWV RNA genome interact with the capsid protein residues. Amino acids involved in the RNA binding are conserved among honeybee iflaviruses, suggesting a putative role of the genome in stabilizing the virion or facilitating capsid assembly.

Release of the virus genome from the capsid is a key step in infection of a cell. The acidic environment, that the virus encounter during cell entry, causes disruption of contacts between DWV capsid proteins, which results in expansion of the virus capsid and formation of pores around icosahedral threefold symmetry axes. This expansion may result in capsid disintegration or genome release though the channels. Understanding the genome release mechanism is a first step for development of compounds able to interupt this essential step in viral life-cycle.

L21

STRUCTURE OF SACBROOD HONEYBEE VIRUS REVEALS EVOLUTION OF CAPSID PROTEIN FEATURES IMPORTANT FOR IFLAVIRUS CELL ENTRY

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Infection by sacbrood virus (SBV) from the family Iflaviridae is lethal to honeybee larvae and causes the collapse of honeybee colonies [1]. Despite the negative effect of the virus on honeybee populations, the structure of its virion and mechanism of its genome delivery are unknown. Here we present the crystal structure of SBV virion and show that it contains sixty copies of a minor capsid protein (MiCP) attached to the virion surface. No similar minor capsid proteins have been previously observed in any of the related picornaviruses. The location of the MiCP coding sequence within the SBV genome indicates that MiCP evolved from a C-terminal extension of a major capsid protein VP3 by the introduction of a cleavage site for a virus protease. Other Iflaviruses (deformed wing virus and slow bee paralysis virus) contain, instead of MiCP, C-terminal extensions of capsid protein VP3 [2,3]. These globular C-terminal domains form protruding crown-like structures at five-fold symmetry axes of these viruses. In contrast, MiCP is positioned closer to three-fold axis, similarly to the “knob” and ”puff” structures in enteroviruses [4,5], rendering the virion surface of SBV smooth.

The exposure of SBV to low pH, which the virus probably encounters during cell entry, induces the formation of pores at threefold and fivefold symmetry axes of the capsid that are 7.8 and 12.5 Å in diameter, respectively. This is in contrast to vertebrate picornaviruses, in which the pores along twofold icosahedral symmetry axes are generally considered the most likely sites for genome release. SBV virions lack VP4 subunits that facilitate the genome delivery of many related dicistro- and picornaviruses [6–8]. However, MiCP subunits induce liposome disruption in vitro, indicating that they are functional analogues of VP4 subunits and enable the virus genome to escape across the endosome membrane into the cell cytoplasm.

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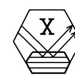

L22

MOLECULAR ARCHITECTURE AND LIFE CYCLE OF FILAMENTOUS VIRUSES

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Potato virus X (PVX) is a member of the family *Alphaflexiviridae* in the genus Potexvirus. It infects several solanaceous crops including potato (it can cause yield losses of up to 15% in some varieties), tomato and tobacco. Symptoms produced by PVX are variable, depending on the strain and host plant. In general, plants often do not exhibit symptoms, but the virus can cause symptoms of mild mottling, chlorosis, mosaic or decreased leaf size. It can be mechanically transmitted.

PVX has a monopartite plus-sense single stranded RNA ((+)ssRNA) of approximately 6.4 kb (Skryabin et al., 1988). PVX virions are flexible rods 515 nm long and 13.5 nm in diameter, consisting of approximately 1350 helically folded identical coat protein (CP) subunits and viral RNA packed between its turns (Tollin and Wilson, 1988).

So far, only low-resolution structure of PVX virion has been determine providing limited information about CP-CP and CP-RNA interactions. The aim of this project is (1) to determine the near-atomic structure of Potato virus X and CP-CP and CP-RNA interacting amino acids and (2) to define the surface exposed regions/loops and test them for possible tolerance of inserted peptides. PVX structure and interacting amino acids was analyzed using cryo-electron microscopy.

Friday, March 23, Session VI

L23

STRUCTURE OF TICK-BORNE ENCEPHALITIS VIRUS AND ITS NEUTRALIZATION BY A MONOCLONAL ANTIBODY

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Tick-borne encephalitis virus (TBEV) causes 13,000 cases of human meningitis and encephalitis annually. However, the structure of the TBEV virion and its interactions with antibodies are unknown. Here, we present cryo-EM structures of the native TBEV virion and its complex with Fab fragments of neutralizing antibody 19/1786. Flavivirus genome delivery depends on membrane fusion that is triggered at low pH. The virion structure indicates that the repulsive interactions of histidine side chains, which become protonated at low pH, may contribute to the disruption of heterotetramers of the TBEV envelope and membrane proteins and induce detachment of the envelope protein ectodomains from the virus membrane. The Fab fragments bind to 120 out of the 180 envelope glycoproteins of the TBEV virion. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments do not lock the E-proteins in the native-like arrangement, but interfere with the process of virus-induced membrane fusion.