

P2 protein for the structural analysis by cryo-electron microscopy to determine the P2 structure and location in the capsid shell. Thus, the P2 proteins are located around 5-fold axis, which reminds us of VP4 spikes of Rotavirus, another member in the *Reoviridae* family. In the case of Rotavirus, the VP4 spikes at the same position as RDV are the major player in the cell entry process [2]. Therefore, they may fold the similar cell entry process in common.

In addition to the *in vitro* study, we determined the *in vivo* supramolecular structure of virus-infected cells by cryo-electron tomography to understand viral life cycle. Studies *in vitro* using purified specimens can not reveal such structural information in the cells and cellular events. When RDV is infected to the host insect cells, formation of protruding tubules composed of viral protein Pns10 can be observed [3,4]. The tubular structure contains RDV particles, and RDV exploits tubules to move into neighboring cells. To understand the viral infection mechanism, viral host cells were cultured on EM grids and infected by RDV. The cells at 3 to 5 days after inoculation were plunged into the liquid ethane and embedded in vitreous ice. Then, the three-dimensional (3D) structure of the tubules containing RDV particles was examined by cryo-electron tomography. The 3D structure clearly visualized the tubular structures containing RDV particles, and their association with cytoskeletal actin and cytoplasmic membrane. These structures gave us new insight into the viral infection mechanism exploiting unique tubular structures.

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Keywords: viral structure, cryo-electron microscopy, cryo-electron tomography

MS02.P04

Acta Cryst. (2011) A67, C229

Structural studies of the DNA end resection complex from *Pyrococcus furiosus*

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Double strand DNA breaks are one of the most serious threats to cell survival and replication. They occur regularly in all cells due to chemical or physical damage, and must be repaired rapidly and accurately to ensure that genetic information is maintained. Two classes of repair system exist, which repair breaks by homologous recombination (HR), or nonhomologous end joining (NHEJ). We study the HR DNA repair system of the archaeon *Pyrococcus furiosus*, which is similar to, albeit simpler than that of higher organisms.

Double strand breaks are first identified by the MR complex, which partially prepares the damaged DNA ends for repair, as well as initiating signaling to the cell that DNA damage has occurred. The break site is then passed to a 500kDa end resection complex consisting of a hexameric HerA helicase ring and a NurA nuclease dimer, which prepares the break site for recombination. The DNA strands adjacent to the break site are unwound by HerA. NurA, a 5' Mn²⁺ dependant nuclease, then removes the 5' strand, leaving a free 3' single strand of DNA for repair by homologous recombination.

We have solved the crystal structure of NurA, which was found to have an RNase H- like fold, with the presumed substrate binding site cleft formed between the two molecules of the dimer. We have

also investigated complexes of NurA with DNA substrates, and have identified both suitable DNA substrates for co-crystallisation, and crystallisation conditions that produce diffracting crystals of the NurA-DNA complex. While efforts to obtain crystals of the HerA-NurA complex have been unsuccessful to date, we are investigating the structure of this using electron microscopy.

We present here the preliminary crystal structure of NurA and our studies into the structure and function of the HerA/NurA resection complex, using a hybrid methods approach consisting of crystallography and EM.

Keywords: nuclease, repair, helicase

MS02.P05

Acta Cryst. (2011) A67, C229-C230

Structural analysis of tubulin binding cofactor complexes by electron microscopy and image processing

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Microtubules are cytoskeletal elements made up of $\alpha\beta$ -tubulin heterodimers that provide structural support for the cell and play important roles in key cellular processes such as cell division, cell motility and intracellular transport. In contrast to other cytoskeletal components (i.e. actin), the α and β -tubulin heterodimer biogenesis pathway involves not only the cytosolic chaperonin CCT for proper folding of the monomer but also a number of tubulin binding cofactors (TBCA-E). Upon one or several rounds of ATPase dependent cycles of CCT activity, quasi-native monomers of tubulin are released from the chaperonin. These folding intermediates subsequently interact with cofactors in a stepwise process that generates assembly competent heterodimers. Furthermore, cofactors D and E can dissociate native heterodimers [1-3] and might also regulate tubulin monomers degradation. Therefore, these proteins would take an important role in the spatial and temporal regulatory pathway of microtubule dynamic instability.

The atomic structure has been solved only for cofactor A [4], the C-terminal glycine-rich cytoskeleton-associated protein domain [5] and the ubiquitin-like β -grasp fold domain of TBCB [6] and the C- (PDB: 2YUH) and N-terminal domains of cofactor C [7]. Complementary studies combining modeling and fold prediction tools [8], have identified the structure of some cofactor domains such as spectrin-like domain in cofactor D and C, leucine-rich repeats in cofactor E, HEAT and Armadillo domains in cofactor D and ubiquitin-like domain (Ubl) in cofactors B and E.

Previously, it has been proposed that some of these domains (i.e. Ubl domains) mediate the interaction between different cofactors and those cofactors with tubulin in a regulated manner that determines microtubule stability or dynamicity. However, no cofactor-cofactor complex or cofactor-substrate complex structures have been solved up to now, and the specific regions of interaction remain to be mapped. Here we propose a preliminary structural analysis by transmission electron microscopy and image processing techniques of different tubulin cofactors, as well as a preliminary docking of several tubulin cofactor domain structures solved by X-ray diffraction.

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