infected by highly pathogenic H5N1 type and H7N7 type avian influenza. There is a clear need for new anti-influenza drugs to combat such viruses, especially as resistance to the current drugs Tamiflu and Relenza is already widespread.

The RNA polymerase is a highly attractive target for new drugs since it plays a variety of essential roles in the viral life-cycle and is more highly conserved than other viral proteins. We concentrated our research efforts on structural studies of the RNA polymerase complex in order to assist structure-based drug design. Influenza virus RNA-dependent RNA polymerase is a hetero-trimer consisting of subunits called PA, PB1, and PB2. PB1 interacts directly with PA and PB2, but PA and PB2 do not. RNA polymerase uses a "cap-snatching" mechanism to produce viral mRNA. Host cell mRNA is cleaved to yield a cap-bearing oligo-nucleotide which can be extended using viral genomic RNA as a template. The cap binding and endonuclease activities are only activated once viral genomic RNA is bound. This requires signaling from the RNA-binding PB1 subunit to the cap-binding PB2 subunit, and the interface between these two subunits is essential for the polymerase activity.

We have defined this interaction surface by protein crystallography, and tested the effects of mutating contact residues on the function of the holo-enzyme[1]. This novel interface is surprisingly small, yet it plays a crucial role in regulating the 250 kDa polymerase complex, and is completely conserved among avian and human influenza viruses.

[1] K. Sugiyama, E. Obayashi, A. Kawaguchi, Y. Suzuki, J.R. Tame, K. Nagata, S.Y. Park. *EMBO J.* **2009**, *28*, 1803-1811.

**Keywords: structure, influenza virus, RNA-polymerase**

## MS29.P09

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**Crystal structure of the viroplasm matrix protein P9-1 of *Rice Black Streaked Dwarf Virus***

Fusamichi Akita,[a] Akifumi Higashiura,[b] Takumi Shimizu,[a] Yingying Pu,[a,c] Mamoru Suzuki,[b] Tamaki Uehara-Ichiki,[a] Takahide Sasaya,[a] Tomitake Tsukihara,[b,d] Atsushi Nakagawa,[b] Toshihiro Omura,[a] *[a]National Agricultural Research Center, 3-1-1 Kan-nondai, Tsukuba, Ibaraki 305-8666, (Japan). [b]Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, (Japan). [c]The National Laboratory of Protein Engineering and Plant Genetic Engineering, Peking-Yale Joint Center for Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, People's Republic of China and [d]Department of Life Science, University of Hyogo, 3-2-1 Koto, Kamighori, Akoh, Hyogo 678-1297, (Japan).* E-mail: fusamiti@affrc.go.jp

*Rice black streaked dwarf virus* (RBSDV), which belongs to the genus *Fijivirus* in the family *Reoviridae*, can replicate both in plants and in an invertebrate insect vector. RBSDV is transmitted to rice, maize, barley and wheat exclusively by the small brown planthopper, after multiplication of the virus in the insect. RBSDV is an icosahedral double-shelled particle of approximately 75 to 80 nm in diameter and contains 10 segments of dsRNA (S1 through S10 in order of their mobility during SDS-PAGE). The genome encodes six putative structural proteins and six putative non-structural proteins. S9 comprises approximately 1.9 kb in size and encodes two ORFs, S9-1 and S9-2, separated by an intercistronic region. The ORF S9-1 at the 5′ end of the S9 encodes a 39.9 kDa protein, namely P9-1. The P9-1 protein of RBSDV accumulates in viroplasms within the cytoplasm of infected cells. They are discrete, punctate viral inclusions, which appear to play an important role in viral morphogenesis and are commonly found in viruses in the family *Reoviridae*. Crystallographic analysis of P9-1 revealed structural features that allow the protein to form dimers via hydrophobic interactions. Each dimer had C-terminal regions, resembling arms, that extended to neighboring dimers, thus uniting sets of four dimers by lateral interaction via hydrophobic interactions to yield cylindrical octamers. Positively and negatively charged patches on the side surfaces of the octamers suggested that octamer might bind laterally to other octamers via electrostatic complementarity. Furthermore, distribution of a positively charged region inside and negatively charged regions on the outer surface of each octamer suggested the three-dimensional stacking of octamer nets, when positively charged areas make contact with negatively charged regions composed of four neighboring octamers. Our structural analysis of P9-1 predicts that this protein has the intrinsic ability to form dimers, octamers, a lateral net of octamers and a three-dimensional viroplasm, as confirmed by the formation of viroplasm-like inclusions when P9-1 was expressed *in vivo* in the absence of other viral proteins and the absence of such inclusions when P9-1 was expressed without its C-terminal arm.

**Keywords: viroplasm, RBSDV, reoviridae**

## MS29.P10

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**Crystallization and preliminary X-ray diffraction analysis of the RNA-dependent RNA polymerase of *Thosea asigna* Virus**

Diego S. Ferrero,[a] José F. Rodríguez,[a] Núria Verdaguer,[b] *[a]Centro Nacional de Biotecnología, CSIC, Calle Darwin nº 3, 28049-Madrid. [b]Institut de Biologia Molecular de Barcelona, CSIC, Parc Científic de Barcelona, Baldiri i Reixac 10, 08028-Barcelona.* E-mail: dferrero@cnb.csic.es

*Thosea asigna* virus (TaV) is a positive-sense, single stranded RNA (ssRNA) virus that belongs to the *Betatetravirus* genera within the *Tetraviridae* family. The genome of TaV consists of an RNA segment (5,700 nucleotides) with two open reading frames, encoding the replicase and capsid protein. The particular TaV replicase does not contain N7-methyl transferase and helicase domains but includes a structurally unique RNA-dependent RNA polymerase (RdRp). All RdRps share a closed "right hand" architecture with fingers, palm and thumb sub-domains, encircling the active site. Sequence analyses identified five ordered sequence motifs (A-B-C-D-E) within the palm subdomain that are conserved in all virus replicases [1]. However, in TaV RdRp motif C is located upstream the motif A. This sequence permutation yields a palm fold in which the canonical structural elements show a non-canonical connectivity (C-A-B-D-E). The permuted palm architecture is also found in double stranded RNA (dsRNA) viruses of the *Birnaviridae* family. Here we report the preliminary crystallographic studies of TaV RdRP. The purified enzyme, produced in insect cells infected with a recombinant baculovirus vector, was crystallized by the sitting drop vapour-diffusion method using PEG 8K and Litium sulphate as precipitants. Two different crystal forms were obtained: native RdRP crystallized in space group P21212 and diffract up to 2.1 Å and the RdRP-Lutetium derivative belong to C2221 space group, diffracting up to 3.0 Å resolution. Data collected at $Lu^{3+}$ absortium maximum ($\lambda$= 1.3404 Å) were suitable for structure determination by single wavelength anomalous dispersion (SAD) technique. The structure of TaV RdRP represents the first structure of a non-canonical RdRP from ssRNA virus. The structural similarities observed between the TaV and Birnavirus RdRps, particularly in the three dimensional organization of their non-canonical palms [2], is a structural evidence that strongly supports the existence of a common ancestor between these apparently unrelated viruses.

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**Keywords: polymerase, virus**

## MS29.P11

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**The structural basis for the essential PA-PB1 subunit interaction in influenza RNA polymerase**

Hisashi Yoshida,[a] Eiji Obayashi,[a] Atsushi Kawaguchi,[b] Kyosuke Nagata,[b] Jeremy R.H. Tame,[a] Sam-Yong Park,[a] [a]*Protein Design Laboratory, Yokohama City University, 1-7-29 Suehiro, Tsurumi, Yokohama, 230-0045, (Japan).* [b]*Department of Infection Biology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, 305-8575, (Japan).* E-mail:h-yosida@tsurumi.yokohama-cu.ac.jp

Influenza A virus is a major human and animal pathogen with the potential to cause catastrophic loss of life. Recently, the emergence of a novel H1N1 viral strain has affected the entire world. Additionally, a highly pathogenic avian influenza caused by H5N1 strain, has a potential of a next pandemic. Most current influenza drug target is haemagglutinin(HA) or neuraminidase(NA), and these protein present at the virion surface. Sixteen different HA subtypes and nine different NA subtypes have been identified. Oseltamivir(Tamiflu) and zanamivir(Relenza) are NA inhibitors, and prevent viral particles being released from infected cells. These drugs have been stocked in world wide, but resistant influenza is already emerging. Another anti-influenza drug amantagine targets the M2 protein of the viral proton channel. However, a single residue change is sufficient to confer resistance. Both oseltamivir and amantadine target proteins with a single known function and substantial sequence variation between viral strains.Therefore, we have to prepare a new method for a new highly pathogenic and oseltamivir-resistant influenza.

The viral RNA polymerase is not yet a target of any approved pharmaceutical, but has recently become a focus for the development of new anti-influenza drugs since it is highly conserved in avian and human influenza. It carries out a number of essential processes in the viral life cycle, many of which remain poorly understood. The three subunits, PB1, PB2 and PA play different roles within the polymerase, and are all essential for viral replication but despite considerable functional analysis relatively little is known about their structure.

Here, we solved the crystal structure of PA-PB1 subunit interaction. The carboxy-terminal domain of PA forms a novel fold, and forms deep, highly hydrophobic groove into which the amino-terminal residues of PB1 can fit by forming a helix.[1] Furthermore, we have found highly conserved residues which are essential for these interactions, and demonstrated that the interruption of these interfaces dramatically reduces viral replication. These interfaces have considerable potential as a drug target sites, which are entirely independent of surface antigen type.

[1] E. Obayashi, H. Yoshida, F. Kawai, Y. Shibayama, A. Kawaguchi, K. Nagata, J.R. Tame, S.Y. Park. *Nature J.* **2008**, *454*, 1127-1131.

**Keywords: structure, influenza virus, RNA-polymerase**

## MS29.P12

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**Crystal structure of measles virus hemagglutinin with its human receptor CD46**

César Santiago,[a] Thilo Stehle,[b] José M. Casasnovas,[a] [a]*Department of Macromolecular Structures and X-ray Crystallography Unit. Spanish National Center of Biotechnology, CSIC. Madrid, (Spain).* [b]*Interfaculty Institute for Biochemistry, University of Tuebingen, Tuebingen, German and Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, (USA).*

Measles virus (MV) remains as a worldwide disease even though there is an effective vaccine available since the sixties. Almost eradicated in most industrialized countries, MV still causes frequent outbreaks, although in developing countries 20 million cases and 300,000 deaths are reported every year annually mainly due to deficiencies in vaccine administration. Therefore, MV has been a priority in World Health Organization vaccination programs for its complete eradication by the end of the 20th century.

Despite all the efforts against MV, little is known about the molecular machinery used by the virus to infect host cells and the early events occurring after virus particle attachment to the host cell. Infection is initiated by attachment of the measles virus hemagglutinin (MV-H), a glycoprotein anchored to the virus envelope, to the host cell receptors CD46 or signaling lymphocyte activation molecule (SLAM).

In this work, we report the crystal structure of MV-H in complex with a CD46 protein spanning the two N-terminal domains. A unique groove at the side of the MV-H β-propeller domain, which is absent in homologous paramyxovirus attachment proteins, engages residues in both CD46 domains. Key contacts involve a protruding loop in the N-terminal CD46 domain that carries two sequential proline residues (PP motif) and penetrates deeply into a hydrophobic socket in MV-H. Viral residues within this extended groove can have certain variability whereas less accessible residues are conserved, which leads to an extended MV tropism by increasing CD46 binding affinity and preserving SLAM binding. The relatively variable and concave surface onto which the CD46 interdomain interface and the SCR2 dock represents an area well suited to accommodate diverse receptor molecules binding to an inaccessible socket in the MV-H protein. Therefore, the use of an extended surface on the side of the β-propeller domain for receptor binding by MV forms the basis for a strategy to extend the virus tissue tropism by receptor-specificity switching.

**Keywords: measles, CD46, hemagglutinin.**

## MS29.P13

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**Studying interactions between vaccinia virus protein a46 and the cellular protein MyD88**

Sofiya Fedosyuk, Tim Skern, *Max F. Perutz Laboratories Vienna Biocenter, Dr. Bohrgasse 9/3, A-1030 Vienna, (Austria).* E-mail: sofiya.fedosyuk@meduniwien.ac.at

Vaccinia virus, closely related to smallpox virus, is a member of the poxvirus family. It is well known for its various strategies to inhibit the immune response of cells of the innate immune system, but the mechanisms of the majority of these important interactions have not been clearly studied. Vaccinia virus encodes a wide range of proteins, which target the pathogen signal transduction pathway from the plasma membrane to the nucleus, and finally prevent production of proinflammatory and antiviral cytokines. Our main goal is to define mechanisms of inhibition of NFκB (the central transcriptional factor during an antiviral response) activation by certain vaccinia proteins by solving their structure and structural mechanisms of interaction with cellular proteins. A profound knowledge of such viral interference strategies will help to understand molecular aspects of viral pathogenesis and to develop novel cell defense approaches. It will give also a deeper insight into cell defense mechanisms.