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.

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Keywords: structure, influenza virus, RNA-polymerase

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

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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

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

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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 (λ= 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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