The Structural Basis for an Essential Subunit Interaction in Influenza Virus RNA Polymerase

Influenza kills more than 250,000 people worldwide every year on average, and estimates of the death toll in the 1918 pandemic range up to 50 million people worldwide [1]. Recent outbreaks of highly pathogenic avian influenza in Asia have rapidly spread across continents, and present vaccines and medication seem unlikely to alleviate greatly any epidemic or pandemic should these viral strains adapt to human hosts [2]. To prepare for such predictable next pandemic produced by mutated highly pathogenic virus like avian one, billions of dollars worth of oseltamivir, which is influenza neuraminidase (NA) inhibitor, were stockpiled, but resistant influenza is already emerging. Another useful anti-influenza drug amantadine targets the M2 protein, however a single residue change is sufficient to confer resistance, which has accordingly risen sufficiently to render the drug useless against many strains. There is therefore ample scope to develop new lead molecules disrupting other processes in the viral life cycle.

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 strains of avian and human influenza [3]. It carries out a number of essential processes in the viral life cycle, but many of these and their regulation remain poorly understood [4]. 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 (Fig. 1). Here, we have solved the crystal structure of a complex formed by N-terminal fragments of PB1 and C-terminal domain of PA at 2.3 Å using **BL41XU** beamline [5].

PA C-terminal domain consists of 13 α helices and 9 β strands and three α helices, α10, α11 and α13, are positioned like the jaws of a clamp, grasping the N terminus of PB1 with the support of a β-hairpin loop made by β8 and β9 (Fig. 2(a)). The interaction region at PB1 lays from 1 to 15 which are visible in the electron density map, whether the rest of peptide of PB1 (16-81) is disordered. PB1 interacts with PA through an array of hydrogen bonds and hydrophobic contacts. Most inter-subunit hydrogen bonds form through main chain atoms of PB1. Residues *Asp 2* to *Asn 4* form anti-parallel β-sheet like interactions with Ile 621 to Glu 623 of PA (Fig. 2(b)). Hydrophobic interactions appear to contribute substantially to the binding energy. *Pro 5* packs between Ile 621 and Trp 706, and *Leu 8* makes contact with the side chains of Met 595, Trp 619, Val 636 and Leu 640 (Fig. 2(c)). Using this model, we designed deletions and point mutations in the C-terminal domain of PA which greatly weaken or abolish PB1 binding, and similarly reduce viral RNA synthesis in human cells (Fig. 3(a) and 3(b)). The levels of vRNA, cRNA, and viral mRNA synthesis were markedly lowered for all the mutants [5]. Further, influenza RNA polymerase shows a high level of sequence conservation across strains, since

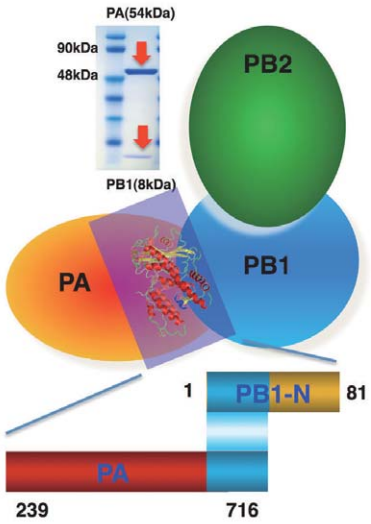

Fig. 1. Schematic diagram of PA, PB1 and PB2 subunits of the influenza RNA polymerase. PB1 binds to C-terminal domain of PA at N-terminus and to N-terminal of PB2 at C-terminus. Purified PA-PB1 complex in this study is analyzed by SDS-PAGE.

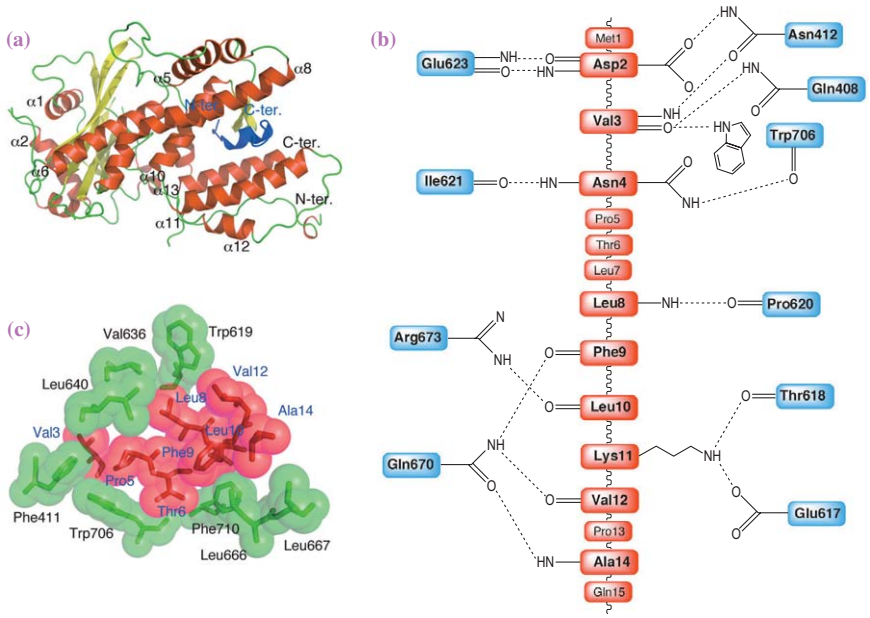

Fig. 2. Crystal structure of the C terminal domain of PA bound to the N terminal peptide of PB1. **(a)** An overall ribbon diagram showing the fold of PA, with helices colored red, strands yellow and coil green. Helices are numbered from the N terminus. PB1 residues are colored dark blue. **(b)** Schematic diagram showing the hydrogen bonds between PA (blue boxes) and PB1 (orange boxes). Black dashed lines indicate hydrogen bonds between 2.4 Å - 3.4 Å in length. **(c)** Space-filling representation, with PA residues show in green and PB1 residues red. Although the interface is largely tightly packed, unfilled space is found adjacent to *Pro 5*, *Thr 6* and *Phe 9* of PB1.

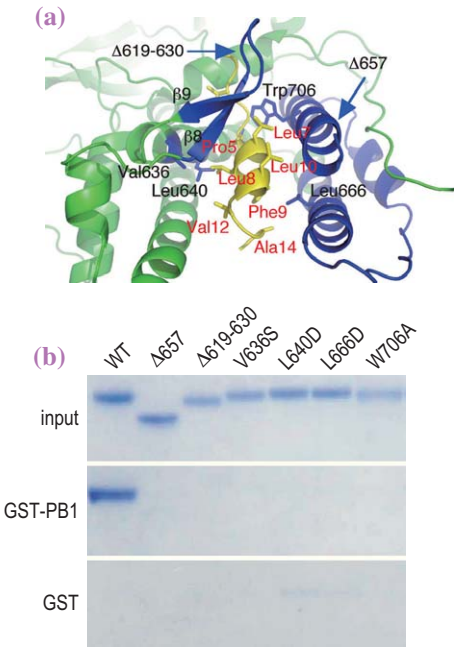

Fig. 3. PB1 binding to PA mutants. **(a)** Cartoon showing the Cα trace of PA in green, with residues selected for mutagenesis or deletion shown in blue. PB1 is shown in yellow (residues labelled in red). Val 636 touches *Leu 8*, Leu 640 lies close to *Leu 8* and *Pro 5*, Leu 666 packs against the side-chain of *Phe 9*, and Trp 706 interacts with *Asn 4*, *Pro 5* and *Thr 6*. **(b)** GST-pull-down assay. Wild-type PA and various mutants were tested for binding to GST-fused to the N-terminal 14 residues of PB1 (middle) or by GST alone as a negative control (bottom).

this PA-PB1 interaction manner is critical for viral replication for all types of influenza A virus, residues involving in the interaction between PA and PB1 are all conserved between those in human (H1N1) for this study and those in highly pathogenic avian (H5N1). From these results, it is concluded that the structure of the highly conserved PB1 binding site on PA presented here provides information to developed into new treatments effective against all types of influenza A virus, including avian strains.

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