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

Influenza A virus is a major human and animal pathogen with the potential to cause catastrophic loss of life. Here we describe the crystal structure of a subunit interface in the viral RNA polymerase, which is the essential enzyme for viral replication. This highly conserved interface between PB1 and PA may have considerable potential as a drug target site entirely independent of surface antigen type.

The recent outbreak of a new swine-related H1N1 influenza virus in Mexico has confused the world economy. However the outbreak is not as severe as was at first feared, because the virus has a low pathogenicity, and although existing vaccines are ineffective, drugs such as the neuraminidase (NA) inhibitors Tamiflu and Relenza have been found to alleviate symptoms greatly [1]. This pandemic, however, has reminded us that we are still in danger from the emergence of new types of influenza virus. This is because humans have no existing immunity to the new types of virus, which thus spread rapidly from person to person. Furthermore, the swine-related H1N1 influenza virus is a hybrid of human, swine and highly pathogenic avian viruses. It is only a matter of time before the emergence of a new hybrid type combining highly pathogenic avian and Tamiflu-resistant human H1N1 viruses. Therefore, we have to prepare now for such killer viruses.

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 [2]. It carries out a number of essential processes in the viral life cycle, but many of these and their regulation remain poorly understood [3]. The three subunits, PB1, PB2 and PA play different roles within

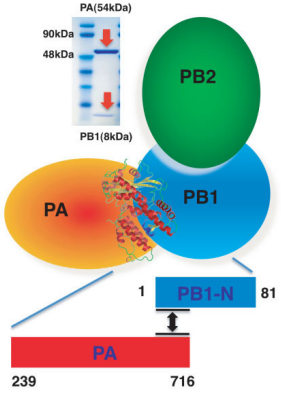

Figure 1
Schematic diagram of the PA, PB1 and PB2 subunits of the influenza RNA polymerase. PB1 binds to the C-terminal domain of PA at the N-terminus and to the N-terminal of PB2 at the C-terminus. The purified PA-PB1 complex in this study was analyzed using SDS-PAGE.

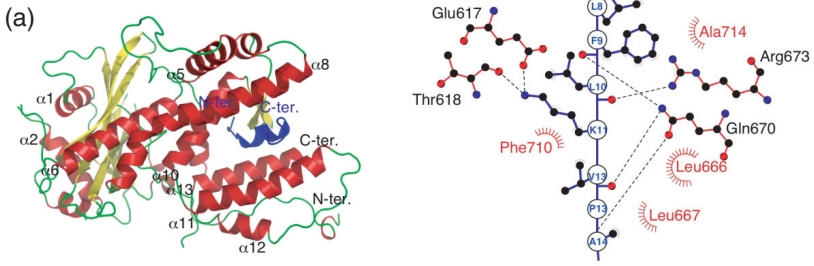

Figure 2
Crystal structure of the C terminal domain of PA bound to the N terminal peptide of PB1. (a) Overall ribbon diagram showing the fold of PA, with helices coloured red, strands yellow and coil green. Helices are numbered from the N terminus, and the PB1 residues are coloured dark blue. (b) Schematic 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. Apolar residues of PA are shown in red as simple dashed arcs to indicate hydrophobic contacts between 3.4-3.9 Å in length.

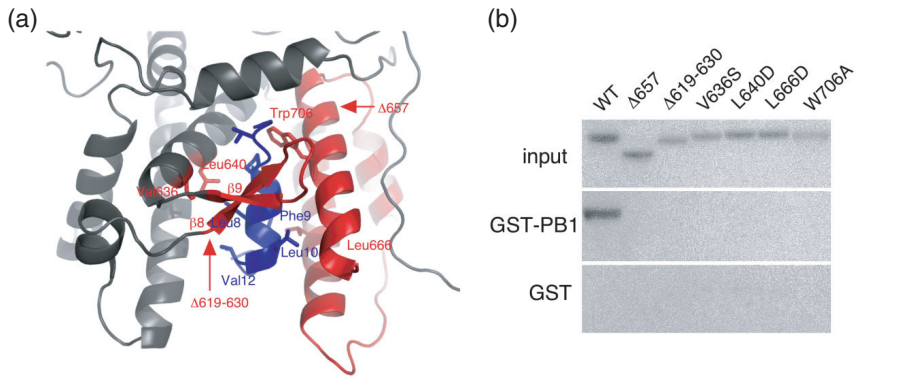

Figure 3
PB1 binding to PA mutants. (a) The ribbon showing the Cα trace of PA in gray, with residues selected for mutagenesis or deletion shown in red (residues labeled in red). PB1 is shown in blue (residues labeled in blue). Val 636 touches Leu 8, Leu 640 lies close to Leu 8 and Pro 5, Leu 666 packs 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).

the polymerase, and are all essential for viral replication, but despite considerable functional analysis relatively little is known about their structures (Fig. 1). Here, we have solved the crystal structure of a complex formed by N-terminal fragments of PB1 and the C-terminal domain of PA at 2.3 Å using BL-5A [4].

The C-terminal domain of PA consists of 13 α helices and 9 β strands. Three of the 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. The rest of the 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 the 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(b)]. 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 (b)]. The levels of vRNA, cRNA, and viral mRNA synthesis were markedly lowered for all the mutants [4]. Further, influenza RNA polymerase shows a high level of sequence conservation across strains, since the manner of the PA-PB1 interaction is critical for viral replication for all types of influenza A virus, and residues involving in the interaction between PA and PB1 are all conserved between those in the human H1N1 virus used 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 useful information for developing new treatments effective against all types of influenza A virus, including avian strains.

REFERENCES

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E. Obayashi, H. Yoshida and S.-Y. Park (Yokohama City Univ.)