Published in final edited form as:
Nat Med. 2009 November ; 15(11): 1253–1254. doi:10.1038/nm1109-1253.

New prospects for the rational design of influenza antivirals

Estanislao Nistal-Villán and **Adolfo García-Sastre**
Estanislao Nistal-Villán and Adolfo García-Sastre are at the Department of Microbiology of Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10028, USA. Adolfo García-Sastre is also affiliated with the Department of Medicine, Division of Infectious Diseases and the Global Health and Emerging Pathogens Institute of Mount Sinai School of Medicine.

Adolfo García-Sastre: adolfo.garcia-sastre@mssm.edu

Abstract

The wide-spread resistance of influenza virus isolates to existing antivirals and the lack of a universal influenza vaccine makes imperative the development of new antivirals to treat influenza, more especially during pandemic years. Two recent publications that determine the crystal structure of the unique endonuclease domain of the influenza virus polymerase open the possibility for the rational design of novel influenza virus inhibitors. [after I edit kanta's piece I will be writing a subhead that also include her commentary, you can comment on that subhead in proofs]

Despite the availability of influenza vaccines, influenza virus continues to be a world-wide public health burden. During typical epidemic years, approximately 36,000 people die and 200,000 are hospitalized just in the US due to severe complications associated with influenza virus infections [1]. These numbers can dramatically increase in severe pandemic years. One of the most extreme that examples of how damaging influenza can be was the 1918–1919 pandemic. This pandemic, caused by an H1N1 virus, resulted in fifty million deaths, mainly among the adult population, and had devastating consequences for humankind [2].

The new H1N1 virus that is causing the first influenza virus pandemic of this century is clearly less virulent than the 1918 virus. But this pandemic episode has given us sobering lessons on our ability to combat pandemic influenza. First of all, it has reminded us of our inability to predict the specific subtype that will start a new pandemic. While most the attention was given mainly to H5, and to some extent to H7, H9 and H2 viruses as potential new pandemic strains, H1 viruses were under the radar because there was no precedent of an influenza pandemic caused by the same subtype virus circulating in humans. However, the large antigenic differences between the new swine-origin H1N1 virus and the previously circulating human H1N1 viruses, responsible for the limiting pre-existing immunity in humans against swine H1N1 viruses, most likely facilitated the species jump from a pig reservoir to humans [3–5]. Moreover, the extended influenza virus surveillance programs in humans allowed us to conclude that the new H1N1 virus started a pandemic with almost no previous period of adaptation to humans, challenging our prospects of producing a vaccine against the new pandemic virus in time to protect the human population. Thus, while a vaccine will soon be available, no vaccination has been possible during the winter season in the Southern Hemisphere, where the new H1N1 virus is prevalent at this time.

During the lagging time needed to produce vaccines, our only weapon against pandemic influenza viruses are antivirals. And even during typical epidemic years, since vaccination does not prevent completely influenza virus infection, antivirals are also critical for the therapeutic treatment of influenza.

Currently, two classes of antivirals have been FDA approved for the treatment of influenza: the M2 channel blockers amantadine and rimantadine, and the neuraminidase (NA) inhibitors oseltamivir and zanamivir. The first class inhibits the ability of the viral M2 protein to pump protons, a function required for viral uncoating during the first steps of viral entry into the host cell. By contrast, the second class inhibits the enzymatic activity of the viral NA protein, needed for the spread of the newly generated influenza virions by the infected host cell. However, a major concern for the use of these antivirals is the wide-spread resistance of circulating influenza viruses. The majority of the human circulating H3N2 viruses are resistant to amantadine and rimantadine, while the human H1N1 viruses of the last season have acquired oseltamivir resistance [6]. The new H1N1 pandemic viruses are resistant to the M2 channel blockers, but sensitive to NA inhibitors. However, resistant isolates have already been described and they may become prevalent under selection pressure. Clearly, novel antivirals are needed to efficiently combat influenza viruses.

A potential new target for the development of novel influenza antiviral compounds is the viral RNA-dependent RNA polymerase. This viral enzyme required for viral RNA replication and transcription has several unique enzymatic properties that make it ideal for the development of specific antivirals. In fact, inhibitors of the influenza polymerase activity have already been described [7], and they have the potential to become new antiviral drugs if proven safe and efficacious in humans. However, one of the hurdles in the rational design of influenza virus RNA polymerase inhibitors has been the lack of detailed structures of its enzymatic domains required for RNA transcription.

The influenza viral RNA polymerase is a heterotrimeric complex composed by three subunits, PB2, PB1 and PA (Fig. 1). During viral mRNA synthesis, the PB1 subunit binds to the promoter of the influenza virus RNA genes formed by the very terminal sequences at the 3' and 5' ends of negative sense viral RNAs, while the PB2 subunit binds to 5' cap structures present in cellular mRNAs. The cellular mRNAs bound to PB2 are subsequently cleaved, generating short capped RNA oligonucleotides that are used as primers for the initiation and elongation of the newly synthesized viral mRNAs. Thus, two enzymatic activities are associated with viral mRNA synthesis by the influenza virus polymerase, an endonuclease that cleaves the cellular mRNAs, a process also referred to as "cap snatching", and an RNA-dependent RNA polymerase that elongates the capped primers using viral RNAs as templates. The PB1 subunit contains the RNA polymerase domain, and for a long time it was believed that the PB1 subunit was also responsible for the endonuclease activity, while the role of the PA subunit in the heterotrimeric complex was unclear.

Only recently, crystal structures of some domains of the viral polymerase associated with protein-protein or protein-RNA interactions have been generated, These include the contact domains between the PB1 and PA proteins [8,9] and between the PB1 and PB2 proteins [10], the PB2 cap-binding domain [11] and the nuclear localization signal of PB2 complexed with importin-α5 [12]. While these complexes represent potential targets for antiviral compounds with the ability to inhibit critical interactions of the viral polymerase, the structure of the whole polymerase complex or of the enzymatic domains of the polymerase will help in the rational design of small molecules that target the active sites of this viral enzyme.

A recent breakthrough was achieved by Yuan et al [13] and Dias et al [14] who independently determined the crystal structure of the first 197 and 209 amino acid residues of the PA subunit, respectively.

Surprisingly, the structure revealed that this domain contains the endonuclease active site of the influenza virus polymerase complex, in contrast to the belief that this activity was located in the PB1 subunit (Fig. 1). The structure also allowed the identification of amino acid residues

conserved in all influenza viruses which bind to metal ions and form the endonuclease active site. Mutations at these amino acids abolished the endonuclease activity of the influenza viral polymerase complex.

This information now provides a structural framework for the identification of small molecule compounds that bind to the endonuclease and inhibit this viral enzyme. Such inhibitors are predicted to prevent viral mRNA synthesis and therefore viral replication. Even more recently, Zhao et al [15], following on the previous PA endonuclease structure, conducted a crystallographic analysis of this domain complexed with nucleoside monophosphates. These studies identified a distinct site inside the catalytic center of the enzyme that binds rUMP, rAMP and TMP but not rGMP or rCMP, highlighting then a nucleotide binding pocket for potential inhibition by small molecule compounds.

The rapid pace by which structures of domains of the influenza virus polymerase have recently been resolved also provides hope that the X-ray structure of the whole heterotrimeric complex of the viral RNA polymerase will be determined in the near future. Such a structure will help in the rational design of inhibitors against the different active domains of the viral polymerase.

The use of inhibitors against multiple domains of the influenza virus polymerase in combination with other antiviral therapies should reduce the probabilities of emergence of resistant strains of influenza viruses. Moreover, the identification of cellular factors required for influenza virus replication may also expand the number of potential targets for the development of antiviral compounds. In this respect, ongoing studies using proteomics and siRNA high throughput screening techniques to determine the subset of cellular proteins and pathways that comprise the functional influenza virus interactome is likely to, in the near future, uncover cellular targets for influenza treatment.

Even if new classes of inhibitors that efficiently reduce viral replication are developed, challenges associated with the time for effective intervention against influenza could still remain. The antivirals already in use are only effective if individuals are treated early during infection, most likely because appearance of disease symptoms only precedes by a few days the peak of influenza viral replication. Thus, any effective therapy for influenza virus requires the development of rapid diagnostic techniques in addition to effective and safe antiviral drugs.

In the absence of an effective influenza virus universal vaccine, more reliable and faster diagnostic methods and new antivirals are required to reduce the burden imposed by epidemic and pandemic influenza.

References

1. Thompson WW, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289:179–186. [PubMed: 12517228]
2. Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918–1920 "Spanish" influenza pandemic. Bull. Hist. Med 2002;76:105–115. [PubMed: 11875246]
3. Garten RJ, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009;325:197–201. [PubMed: 19465683]
4. Itoh Y, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 2009;460:1021–1025. [PubMed: 19672242]
5. Hancock K, et al. Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus. N. Engl. J. Med. 2009 on line.
6. Moscona A. Global transmission of oseltamivir-resistant influenza. N. Engl. J. Med 2009;360:953–956. [PubMed: 19258250]
7. Beigel J, Bray M. Current and future antiviral therapy of severe seasonal and avian influenza. Antiviral Res 2008;78:91–102. [PubMed: 18328578]

8. Obayashi E, et al. The structural basis for an essential subunit interaction in influenza virus RNA polymerase. Nature 2008;454:1127–1131. [PubMed: 18660801]

9. He X, et al. Crystal structure of the polymerase PA(C)-PB1(N) complex from an avian influenza H5N1 virus. Nature 2008;454:1123–1126. [PubMed: 18615018]

10. Sugiyama K, et al. Structural insight into the essential PB1-PB2 subunit contact of the influenza virus RNA polymerase. Embo J 2009;28:1803–1811. [PubMed: 19461581]

11. Guilligay D, et al. The structural basis for cap binding by influenza virus polymerase subunit PB2. Nat. Struct. Mol. Biol 2008;15:500–506. [PubMed: 18454157]

12. Tarendeau F, et al. Structure and nuclear import function of the C-terminal domain of influenza virus polymerase PB2 subunit. Nat. Struct. Mol. Biol 2007;14:229–233. [PubMed: 17310249]

13. Yuan P, et al. Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. Nature 2009;458:909–913. [PubMed: 19194458]

14. Dias A, et al. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature 2009;458:914–918. [PubMed: 19194459]

15. Zhao C, et al. Nucleoside monophosphate complex structures of the endonuclease domain from the influenza virus polymerase PA subunit reveal the substrate binding site inside the catalytic center. J. Virol 2009;83:9024–9030. [PubMed: 19587036]

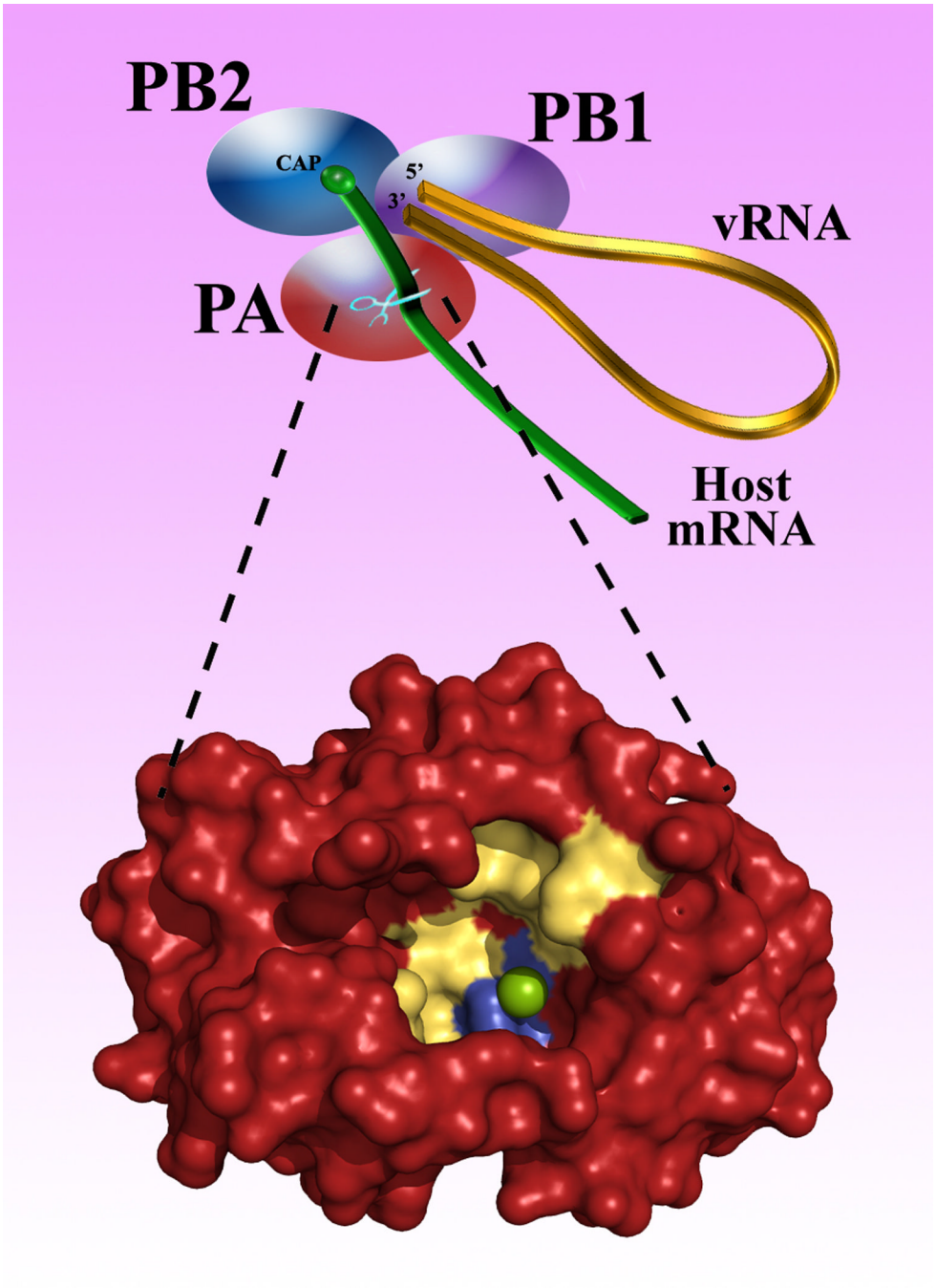

Figure 1. New antiviral target

Schematic representation of the initiation of viral mRNA synthesis by the influenza virus RNA polymerase and of the structure of its endonuclease active site. The polymerase is comprised by three subunits, PB1, PB2 and PA. The PB1 subunit binds to the 5' and 3'ends of the viral RNA template (in yellow), while the PB2 subunit binds to the 5' capped ends of a host cellular mRNA (in green). Cleavage of the cellular mRNA mediated by the endonuclease domain of the PA subunit generates the primers used by the RNA-dependent RNA polymerase domain of the PB1 subunit for viral mRNA synthesis. The structure of the endonuclease active site is represented in the bottom of the figure (PDB accession # 3EBJ), and it revealed a nucleoside monophosphate binding pocket (in yellow) where amino acid residues (in blue) binding to Mg

++ (in green) are also located [13–15]. This pocket represents a new target for the development of small molecule inhibitors.