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Review

Influenza virus polymerase: Functions on host range, inhibition of cellular response to infection and pathogenicity

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ABSTRACT

The viral polymerase is an essential complex for the influenza virus life cycle as it performs the viral RNA transcription and replication processes. To that end, the polymerase carries out a wide array of functions and associates to a large number of cellular proteins. Due to its importance, recent studies have found numerous mutations in all three polymerase protein subunits contributing to virus host range and pathogenicity. In this review, we will point out viral polymerase polymorphisms that have been associated with virus adaptation to mammalian hosts, increased viral polymerase activity and virulence. Furthermore, we will summarize the current knowledge regarding the new set of proteins expressed from the viral polymerase genes and their contribution to infection. In addition, the mechanisms used by the virus to counteract the cellular immune response in which the viral polymerase complex or its subunits are involved will be highlighted. Finally, the degradative process induced by the viral polymerase on the cellular transcription machinery and its repercussions on virus pathogenicity will be of particular interest.

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1. Introduction

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The influenza A virus (IAV) is an important respiratory human pathogen causing yearly recurrent seasonal epidemics with an average global burden of >600 million cases (www.who.int). In rare instances IAV can also spread from its natural zoonotic reservoirs (aquatic birds) to cross species barriers and transmit to humans where it can evolve into strains that cause diseases ranging from mild to severe, with occasional widespread distribution known as pandemic. In the past century, the most devastating pandemic took place in 1918–1920 (also known as the "1918 flu" or "Spanish flu"), infecting hundreds of millions and killing between 20 and 50 million people worldwide (Johnson and Mueller, 2002; Taubenberger and Morens, 2006). Two additional pandemic events originating in Asia took place during the second half of the 20th century, the so-called Asian H2N2 pandemic of 1957 (1 million deaths) and the Hong Kong H3N2 pandemic in 1968 (1 million deaths). Although of relatively low virulence, the recent H1N1 influenza 2009 pandemic of swine origin emerged unexpectedly in Mexico to spread around the world in just a few months. IAV naturally infects wild aquatic birds making up an extremely heterogeneous population which includes many possible combinations between the two surfaces glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA); a total of 18 different HA and 11 NA have been recognized. At present there are concerns that avian influenza strains, including the highly pathogenic influenza viruses H5N1 and the novel H7N9 subtypes not yet capable of spreading from one human to another, could adapt and become more easily transmissible among humans.

The ability of influenza A viruses to infect a variety of hosts is based on their genetic diversity due to two main reasons: (i) their RNA polymerase is error-prone, and (ii) they contain a segmented genome, which allows for exchange of RNA segments between genotypically diverse influenza viruses. These features lead to the rapid generation of novel strains and subtypes and thus contribute to the constant threat that newly emerging and re-emerging influenza viruses pose to the human population.

1.1. Structure and function of the influenza polymerase

IAV, a member of the *Orthomyxoviridae* family, possesses a negative-sense single-stranded RNA genome (vRNA) divided into eight segments. vRNAs are protected in a structure known as viral ribonucleoprotein (vRNP), in which the RNA strand is wrapped by the nucleoprotein (NP), and a single viral polymerase complex interacts with the complementary 3′ and 5′ genomic end sequences. Recent cryo-EM reconstructions of vRNPs obtained from different sources show a double-helical stem structure in which the NP proteins and the protected viral RNA form two anti-parallel strands (Arranz et al., 2012; Moeller et al., 2012). Both strands are connected by a short loop at one end of the particle and interact with the viral polymerase at the other end. The incoming parental vRNPs are released into the cytoplasm of the infected host cell and then quickly transported into the nucleus. IAV is a rare RNA-genome virus in that expression and amplification of viral genomes take place inside the infected cell nucleus and it therefore heavily depends on host nucleocytoplasmic trafficking and nuclear functions. Within the host nucleus, cellular insoluble fractions, such as nuclear matrix and chromatin structures, have been shown to encompass part of the viral polymerase transcription and replication activity and act as a platform for the release of progeny vRNPs outside the nucleus (Chase et al., 2011; Garcia-Robles et al., 2005; Lopez-Turiso et al., 1990; Takizawa et al., 2006).

The IAV genome encodes for 10 major proteins, although alternative protein products have been characterized from several genome segments (see below). The complete coding capacity of the IAV genome is far from known and processes like splicing, protein truncations, and the use of alternative initiation codons or overlapping frames are known to increase the diversity of proteins generated during infection. The widespread synthesis of small viral non-coding RNAs with largely unknown roles in the outcome of infection further complicates the understanding of IAV full expression capacity (Perez et al., 2010, 2012). The three largest viral genome segments encode for the polymerase heterotrimeric complex, responsible for the RNA-dependent RNA polymerase activity of the virus (Fig. 1). The three polymerase subunits, named PA, PB1 and PB2, together with the nucleoprotein, form the minimum set of viral proteins required for viral RNA transcription and replication (reviewed in (Fodor, 2013; Resa-Infante et al., 2011)). Viral transcription depends on primers of host origin obtained through a cap-snatching process targeting newly synthesized host pre-mRNAs (Krug et al., 1979). The viral positive-sense mRNA also includes a 3′ polyA tail generated by repetitive polymerization of a polyU track on the genomic vRNP, thus creating a transcript that is structurally undistinguished from host mRNAs. The vRNP undergoing transcription is processed by a cis-acting polymerase (Jorba et al., 2009). The requirement of newly synthesized cellular pre-mRNAs as a source of cap-oligonucleotides for viral transcription initiation involves a functional association between the viral and the cellular transcription machineries, which has significant consequences for viral outcome (see below).

A switch from viral transcription to replication is required during a successful infectious cycle. The action of viral short virion RNAs (svRNAs) and the availability of cellular nucleotides and newly synthesized viral polymerase and NP, have been involved in this process (Jorba et al., 2009; Perez et al., 2010; Vreede and Brownlee, 2007; Vreede et al., 2004). The viral replication step is initiated early during infection with the synthesis of the cRNA, a complete unpolyadenylated positive-polarity copy of the vRNA (Hay et al., 1977). cRNAs within the infected host cell form cRNPs structures, and serve as templates for the synthesis of new vRNPs which are ready for either further rounds of viral gene expression or their transportation outside the nucleus and subsequent encapsidation. As opposed to what happens with the viral mRNA, the production of cRNAs is only started after viral protein synthesis has begun and seems to require a soluble trans-acting polymerase different from the one resident in the RNP (Hay et al., 1977; Jorba et al., 2009).

Basic protein 1, PB1, is the core of the complex, the most conserved of the polymerase subunits, and contains the enzymatic motifs needed for RNA polymerization activity (Kobayashi et al., 1996). The PB2 subunit has a key role in viral transcription due to its recognition and binding of host 5′ mRNA cap structures generated by the cellular transcription machinery (Blaas et al., 1982; Braam et al., 1983). Acidic protein PA has an endonucleolytic activity needed for the viral cap-snatching process (Dias et al., 2009; Yuan et al., 2009). In recent years some parts of the three proteins have been structurally characterized including important

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