Transcription and Replication of Influenza A Virus

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Leo Poon was a scholar of Croucher Foundation and had his doctoral training in Sir William Dunn School of Pathology in University of Oxford between 1996-1999. After his graduation, he returned to Hong Kong and worked as a postdoctoral fellow in the Chinese University of Hong Kong. He joined the Department of Microbiology of this faculty in 2001 as a research assistant professor and became an assistant professor in 2003.

Dr Poon has diverse research interests, ranging from studying very basic biology of RNA viruses to developing molecular diagnostic tests for infectious diseases. He has a long term interest on the transcription and replication of influenza viral RNA. He is also interested in using molecular techniques to understand the pathogenesis of avian influenza viruses of zoonotic potential. In 2003, Dr Poon was involved in the discovery of SARS coronavirus as the aetiology of the disease and he is the one who first deduced the viral sequence in the group. These findings allowed him to develop several useful molecular tests for the diagnosis of SARS. The identification of SARS coronavirus in humans and animals prompted him to hunt novel viruses in wildlife. Recently, Dr Poon has identified a novel group 1 coroanvirus in bat species (*Miniopterus spp.*).

In eukaryotic cells, messenger RNA production requires synthesis of a pre-mRNA by RNA polymerase II (Pol II) and processing of the nascent precursor by 5' capping, splicing of introns, and 3' cleavage/polyadenylation to make mature mRNA. These events occur cotranscriptionally and it is well known that the C-terminal heptad repeat domain (CTD) of RNA pol II facilitates the above RNA maturation processes. Recent evidences further demonstrated that factors involve in capping, splicing and polyadenylation interact with the CTD, suggesting that RNA pol II plays an important role in mRNA maturation.

The genome of influenza A virus contains 8 RNA segments of negative polarity. Each virion RNA (vRNA) can be used as a template for transcription and replication to generated viral mRNA and complementary RNA (cRNA), respectively. Interestingly, although viral mRNA from the M and NS gene of influenza virus are entirely generated from a viral polymerase complex, these viral mRNA molecules utilize host's major splicing machinery to generate spliced mRNA. Little is known about how the viral mRNA can undergo splicing in the absence of direct involvement of host's RNA pol II. In this study, we elucidated the possible role of 5' end of viral intron for influenza mRNA splicing. In particular, the effect of mutating the two nucleotides (GU), which are invariable in introns undergo U2-type splicing pathway, at the 5' end of viral intron were studied. First, an in vivo system that mimics to the viral replication was used to elucidate the role of these two nucleotides for viral mRNA splicing. In addition, we also used reverse genetic systems to generate recombinant viruses with silent mutations at 5' splicing site of M gene. Strikingly, we were able to rescue M2 knockout mutants. These mutants had attenuated phenotypes, suggesting this novel approach might be used for live attenuated virus vaccines production. Finally, our analysis of M and NS gene suggested that there might be a selection pressure exerted by the host towards the 5' splicing site of influenza viral mRNA.