Characterization of viral and host modulators of influenza virus RNA synthesis machinery using molecular and cellular approaches

Abstract

Influenza virus RNA synthesis is a complex multistep process, which needs to be executed in a highly regulated manner. In addition to viral polymerase and nucleoproteins, influenza virus largely depends upon a plethora of host factors for the smooth execution of these processes inside the host cells. Thus, a greater understanding of viral and host determinants in temporal and global regulation of viral RNA synthesis could be helpful in developing more effective countermeasures against influenza virus. Bat influenza viruses have been recently discovered and their epizootic or zoonotic transmission potential remains largely uncharacterized. This thesis includes systematic structural and bioinformatic analysis of viral polymerase subunit proteins to identify species-specific signatures that may serve as replication determinants of bat influenza virus in bat and non-bat host species. Functional screening of shortlisted residues identified PB2-282nd residue along with previously known host adaptive PB2- 627th residue as a critical determinant of viral polymerase function. Introduction of bat virus-specific serine residues at these specific positions of the PB2 of non-bat infecting influenza A/H1N1/WSN/1933 virus significantly reduce its replication efficiency. On the other hand, introduction of human or avian virus-specific glutamic acid at 282nd and lysine at 627th position enhanced the bat virus polymerase function in human cells. Furthermore, the PB2-282 resides within a highly conserved three amino acid "S279-E282-S286" motif which is critical for the intrinsic polymerase function of the virus. Together these data identified the PB2-282 and the associated "S-E-S" motif as one of the critical regulators of influenza virus polymerase function which may regulate replication and adaptation of bat influenza viruses in non-bat host species. The thesis also contains extensive biochemical experiments to map the interaction of influenza virus polymerase and host protein kinase c delta (PKCδ). Experiments utilizing a structure-guided truncation library of the PB2 protein revealed that the "mid domain" harboring the E-282nd residue and "627 domain" harboring the K-627th residue are instrumental for interaction with PKCδ. Interestingly, the adaptive serine residues in viral polymerase are potential phosphorylation sites and reside within the interaction interface with the host determinant PKCδ, a kinase, hence indicating possible cross-talks between them.