Chapter One

Introduction
Emerging infectious diseases represent substantial threats to global health and Acquired Immunodeficiency Syndrome (AIDS) ranks as one of the most important infectious diseases that humans are facing in the 21st century. Since its clinical description less than two decades ago, AIDS has resulted in the deaths of more than 20 million people worldwide. Human immunodeficiency virus type-1 (HIV-1) and type-2 (HIV-2), the etiological agents for AIDS, have infected more than 50 million individuals worldwide, and the new infections increase at the rate of 6 million per year. Although sub-Saharan Africa remains the global epicenter, rates of infection have increased in recent times in the former Soviet Union and parts of south and south-east Asia, including India and China. Combination of anti-retroviral therapy has afforded many people clinical relief but vast majority of the infected people worldwide do not have access to these agents.

The AIDS viruses HIV-1, HIV-2 and the closely related simian immunodeficiency viruses (SIVs) belong to lentivirus subfamily of retroviruses. These viruses have remarkable properties of insidious disease induction, persistence, latency, variation, recombination and escape from immune and drug pressures. HIV-1 and HIV-2 are the result of zoonotic transmission of SIVcpz in chimpanzees (Pan troglodytes troglodytes) from West central Africa and SIVsm in sooty mangabeys (Cercocebus atys) from West Africa respectively. At least three different zoonotic jumps from chimpanzees into humans led to the disproportionate spread of HIV-1 groups M, O and N. In addition humans have apparently picked up as many as seven lineages of viruses from sooty mangabeys resulting in HIV-2 subtypes A through G. HIV-1, HIV-2 and SIV have a ~10kb RNA genome, and carry three structural genes (gag, pol and env) and two regulatory genes (rev and tat) that are essential for replication. These viruses also have accessory genes that are not essential for
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replication in vitro, but can dramatically alter the course and severity of infection, replication and disease progression in vivo. Vif, vpr and nef are present in HIV-1, HIV-2 and related SIV where as vpu is present only in HIV-1 and related SIVcpz and SIVgsn. In contrast, Vpx is present only in HIV-2 and related SIVsm, SIVrcm, and SIVmac. Containment of AIDS epidemic will require an effective vaccine against HIV infection. Lack of animal model has hampered the vaccine development, even though far from perfect non-human primates offer the only animal model due to similarity in physiology and natural course of infection. SIV naturally infect a variety of non-human primates. Although SIV share many structural and biological properties with human immunodeficiency viruses, they do not seem to induce AIDS in their natural hosts. In contrast, SIV from sooty mangabeys (SIVsm) induces an immunodeficiency syndrome very similar to AIDS when it is experimentally inoculated into Asian monkey species such as rhesus macaques. Non-human primate models continue to provide an important tool for assessing the extent of protective immunity induced by various immunization strategies.

A critical step in the process of retrovirus infection is the transfer of viral DNA into the nucleus of the infected cells. Lentiviruses like HIV/SIV are capable of infecting non-dividing cells such as terminally differentiated macrophages and memory T-cells, which are important for in vivo viral dissemination and persistence. In contrast, prototypic retroviruses do not replicate efficiently in non-dividing cells. After viral entry into the cell, genomic HIV/SIV RNA is reverse transcribed into linear double-stranded DNA, which remains associated with a nucleoprotein complex called preintegration complex (PIC). The viral PICs are then imported into the nucleus through the nuclear pore complex (NPC) via an active mechanism within four to six hours after infection. One cis-acting element, central DNA flap and at least three
proteins namely integrase, Gag matrix protein and Vpr protein have been identified as possible mediators of the nuclear import of the HIV-1 PIC. Interestingly HIV-2/SIVsm/SIVmac contains a *vpr* gene as well as evolutionarily related *vpx* gene. Vpr induces cell cycle arrest at G2 stage whereas Vpx was found to be the major determinant involved in the nuclear transport of PIC. Vpx is also essential for efficient *in vivo* dissemination and spread of SIVsm following mucosal and intravenous infection of macaques. Vpx mutant SIVsm is significantly reduced in its ability to replicate in non-dividing target cells such as monocyte derived macaque macrophages. Vpx is packaged into the viral particles via its interaction with structural protein Gag. Based on such late expression during virus production and early availability during initial infection, it has been proposed that Vpx is involved in the efficient import of viral DNA into the nuclear compartment of non-dividing target cells. But the domains required for Vpx nuclear import and for the efficient virus replication in non-dividing cells have so far not been reported. Also, the mechanism by which Vpx regulates the nuclear import of HIV-2/SIVsm remains unknown.

The present study was designed to address the following issues:
1. Identification and characterization of signal(s) involved in Vpx nuclear localization.
2. Elucidating the mechanism(s) that regulate the nuclear transport of Vpx.
3. To define the signal(s) and the mechanism(s) of Vpx packaging into virus particles.