alternate splicing factor/splicing factor 2 (ASF/SF2)and its associated p32 protein.

Transport of the incompletely spliced viral transcripts to the cytoplasm depends on an adequate supply of Rev.Rev is a small shuttling protein that binds a complex RNA stem-loop termed the Rev response element (RRE), which is located in the *env* gene. Rev binds first with high affinity to a small region of the RRE termed the stem-loop IIB.This binding leads to the multimerization of Rev on the remainder of the RRE. In addition to a nuclear localization signal, Rev contains a leucine-rich nuclear export sequence (NES).Of note, the study of Rev was the catalyst for the discovery of such NES in many cellular proteins and led to identification of the complex formed between CRM1/exportin-1 and this sequence.

The nuclear export of this assembly (viral RNA transcript, Rev, and CRM1/exportin 1) depends critically on yet another host factor, RanGTP. Ran is a small guanine nucleotide-binding protein that switches between GTP- and GDP-bound states. RanGDP is found predominantly in the cytoplasm because the GTPase activating protein specific for Ran (RanGAP) is expressed in this cellular compartment. Conversely, the Ran nucleotide exchange factor, RCC1, which charges Ran with GTP, is expressed predominantly in the nucleus. The inverse nucleocytoplasmic gradients of RanGTP and RanGDP produced by the subcellular localization of these enzymes likely plays a major role in determining the directional transport of proteins into and out of the nucleus. Outbound cargo is only effectively loaded onto CRM1/exportin-1 in the presence of RanGTP. However, when the complex reaches the cytoplasm, GTP is hydrolyzed to GDP, resulting in release of the bound cargo. The opposite relationship regulates the nuclear import by importins alpha and beta, where nuclear RanGTP stimulates cargo release.

For **HIV** infection to spread, a balance between splicing and transport of viral mRNA species must be achieved. If splicing is too efficient, then only the multiply spliced transcripts appear in the cytoplasm. Although rewarded, the regulatory proteins encoded by multiply spliced transcripts are insufficient to support full viral replication. However, if splicing is impaired, adequate synthesis of Tat, Rev, and Nef will not occur. In many non-primate cells, **HIV** transcripts may be overly spliced, effectively preventing viral replication in these hosts.

HIV Replication

In contrast to Tat and Rev, which act directly on viral RNA structures, Nef modifies the environment of the infected cell to optimize viral replication.The absence of Nef in infected monkeys and humans is associated with much slower clinical progression to AIDS.This virulence caused by Nef appears to be associated with its ability to affect signaling cascades, including the activation of T-cell antigen receptor,and to decrease the expression of CD4 on the cell surface.Nef also promotes the production and release of virions that are more infectious.Effects of Nef on the PI3-K signaling cascade--which involves the guanine nucleotide exchange factor Vav, the small GTPases Cdc42 and Rac1, and p21-activated kinase PAK--cause marked changes in the intracellular actin network, promoting lipid raft movement and the formation of larger raft structures that have been implicated in T-cell receptor

signaling.Indeed, Nef and viral structural proteins colocalize in lipid rafts.Two other **HIV** proteins assist Nef in downregulating expression of CD4. The envelope protein gp120 binds CD4 in the endoplasmic reticulum, slowing its export to the plasma membrane,(and Vpu binds the cytoplasmic tail of CD4, promoting recruitment of TrCP and Skp1p.These events target CD4 for ubiquitination and proteasomal degradation before it reaches the cell surface.

Nef acts by several mechanisms to impair immunological responses to **HIV**. In T cells, Nef activates the expression of FasL, which induces apoptosis in bystander cells that express Fas, thereby killing cytotoxic T cells that might otherwise eliminate **HIV**-1 infected cells. Nef also reduces the expression of MHC I determinants on the surface of the infected celland so decreases the recognition and killing of infected cells by CD8 cytotoxic T cells. However, Nef does not decrease the expression of HLA-C,(which prevents recognition and killing of these infected cells by natural killer cells.

Nef also inhibits apoptosis. It binds and inhibits the intermediate apoptosis signal regulating kinase-1 (ASK-1)that functions in the Fas and TNFR death signaling pathways and stimulates the phosphorylation of Bad leading to its sequestration by 14-3-3 proteins.Nef also binds the tumor suppressor protein p53, inhibiting another potiential initator of apoptosis. Via these different mechanisms, Nef prolongs the life of the infected host cell, thereby optimizing viral replication.

Other viral proteins also participate in the modification of the environment in infected cells. Rev-dependent expression of Vpr induces the arrest of proliferating infected cells at the G2/M phase of the cell cycle. Since the viral LTR is more active during G2, this arrest likely enhances viral gene expression.These cell-cycle arresting properties involve localized defects in the structure of the nuclear lamina that lead to dynamic, DNA-filled herniations that project from the nuclear envelope into the cytoplasm.Intermittently, these herniations rupture, causing the mixing of soluble nuclear and cytoplasmic proteins.Either alterations in the lamina structure or the inappropriate mixing of cell cycle regulators that are normally sequestered in specific cellular compartments could explain the G2 arresting properties of Vpr.

Viral Assembly

New viral particles are assembled at the plasma membrane. Each virion consists of roughly 1500 molecules of Gag and 100 Gag-Pol polyproteins, two copies of the viral RNA genome, and Vpr. Several proteins participate in the assembly process, including Gag polyproteins and Gag-Pol, as well as Nef and Env. A human ATP-binding protein, HP68 (previously identified as an RNase L inhibitor), likely acts as a **molecular** chaperone, facilitating conformational changes in Gag needed for the assembly of viral capsids. In primary CD4 T lymphocytes, Vif plays a key but poorly understood role in the assembly of infectious virions. In the absence of Vif, normal levels of virus are produced, but these virions are noninfectious, displaying arrest at the level of reverse transcription in the subsequent target cell. Heterokaryon analyses of cells formed by the fusion of nonpermissive (requiring Vif for