

LANDMARKS:

HIV GENOMIC STRUCTURAL ELEMENTS

- LTR** Long terminal repeat, the DNA sequence flanking the genome of integrated proviruses. It contains important regulatory regions, especially those for transcription initiation and polyadenylation.
- TAR** Target sequence for viral transactivation, the binding site for Tat protein and for cellular proteins; consists of approximately the first 45 nucleotides of the viral mRNAs in HIV-1 (or the first 100 nucleotides in HIV-2 and SIV.) TAR RNA forms a hairpin stem-loop structure with a side bulge; the bulge is necessary for Tat binding and function.
- RRE** Rev responsive element, an RNA element encoded within the env region of HIV-1. It consists of approximately 200 nucleotides (positions 7327 to 7530 from the start of transcription in HIV-1, spanning the border of gp120 and gp41). The RRE is necessary for Rev function; it contains a high affinity site for Rev; in all, approximately seven binding sites for Rev exist within the RRE RNA. Other lentiviruses (HIV-2, SIV, visna, CAEV) have similar RRE elements in similar locations within env, while HTLVs have an analogous RNA element (RXRE) serving the same purpose within their LTR; RRE is the binding site for Rev protein, while RXRE is the binding site for Rex protein. RRE (and RXRE) form complex secondary structures, necessary for specific protein binding.
- CRS** Cis-acting repressive sequences postulated to inhibit structural protein expression in the absence of Rev. One such site was mapped within the pol region of HIV-1. The exact function has not been defined; splice sites have been postulated to act as CRS sequences.
- INS** Inhibitory/Instability RNA sequences found within the structural genes of HIV-1 and of other complex retroviruses. Multiple INS elements exist within the genome and can act independently; one of the best characterized elements spans nucleotides 414 to 631 in the gag region of HIV-1. The INS elements have been defined by functional assays as elements that inhibit expression posttranscriptionally. Mutation of the RNA elements was shown to lead to INS inactivation and up regulation of gene expression.

GENES AND GENE PRODUCTS

- GAG** The genomic region encoding the capsid proteins (group specific antigens). The precursor is the p55 myristylated protein, which is processed to p17 (MA_{matrix}), p24 (CA_{capsid}), p7 (NucleoCA_{capsid}), and p6 proteins, by the viral protease. Gag associates with the plasma membrane where the virus assembly takes place. The 55 kDa Gag precursor is called assemblin to indicate its role in viral assembly.
- POL** The genomic region encoding the viral enzymes protease, reverse transcriptase and integrase. These enzymes are produced as a Gag-pol precursor polyprotein, which is processed by the viral protease; the Gag-pol precursor is produced by ribosome frameshifting at the C-terminus of gag.
- ENV** Viral glycoproteins produced as a precursor (gp160) which is processed to give a noncovalent complex of the external glycoprotein gp120 and the transmembrane glycoprotein gp41. The mature gp120-gp41 proteins are bound by non-covalent interactions and are associated as a trimer on the cell surface. A substantial amount of gp120 can be found released in the medium. gp120 contains the binding site for the CD4 receptor, and the seven transmembrane domain chemokine receptors that serve as co-receptors for HIV-1.
- TAT** Transactivator of HIV gene expression. One of two essential viral regulatory factors (Tat and Rev) for HIV gene expression. Two forms are known, Tat-1 exon (minor form) of 72 amino acids and Tat-2exon (major form) of 86 amino acids. Low levels of both proteins are found in persistently infected cells. Tat has been localized primarily in the nucleolus/nucleus by immunofluorescence. It acts by binding to the TAR RNA element and activating transcription

initiation and/or elongation from the LTR promoter. It is the first eukaryotic transcription factor known to interact with RNA rather than DNA and may have similarities with prokaryotic anti-termination factors. Extracellular Tat can be found and can be taken up by cells in culture.

- REV** The second necessary regulatory factor for HIV expression. A 19 kD phosphoprotein, localized primarily in the nucleolus/nucleus, Rev acts by binding to RRE and promoting the nuclear export, stabilization and utilization of the viral mRNAs containing RRE. Rev is considered the most functionally conserved regulatory protein of lentiviruses. Rev cycles rapidly between the nucleus and the cytoplasm.
- VIF** Viral infectivity factor, a basic protein of typically 23 kD. Promotes the infectivity but not the production of viral particles. In the absence of Vif the produced viral particles are defective, while the cell-to-cell transmission of virus is not affected significantly. Found in almost all lentiviruses, Vif is a cytoplasmic protein, existing in both a soluble cytosolic form and a membrane-associated form. The latter form of Vif is a peripheral membrane protein that is tightly associated with the cytoplasmic side of cellular membranes. Some recent observations suggest that Vif functions late in replication to modulate assembly, budding, and/or maturation the N-terminal half of Vif (N'-Vif) specifically interacts with viral protease.
- VPR** Vpr (viral protein R) is a 96-amino acid (14 kd) protein, which is incorporated into the virion. It interacts with the p6 gag part of the Pr55 gag precursor. Vpr detected in the cell is localized to the nucleus. Proposed functions for Vpr include the targeting the nuclear import of preintegration complexes, cell growth arrest, transactivation of cellular genes, and induction of cellular differentiation. It is found in HIV-1, HIV-2, SIVmac and SIVmd. It is homologous to the vpx protein.
- VPU** Vpu (viral protein U) is unique to HIV-1 and SIVcpz, a close relative of HIV-1. There is no similar gene in HIV-2 or other SIVs. Vpu is a 16-kd (81-amino acid) type I integral membrane protein with at least two different biological functions: (a) degradation of CD4 in the endoplasmic reticulum, and (b) enhancement of virion release from the plasma membrane of HIV-1-infected cells. Env and Vpu are expressed from a bicistronic mRNA. Vpu probably possesses an N-terminal hydrophobic membrane anchor and a hydrophilic moiety. It is phosphorylated by casein kinase II at positions Ser52 and Ser56. Vpu is involved in env maturation and is not found in the virion. Vpu has been found to increase susceptibility of HIV-1 infected cells to Fas killing.
- NEF** A multifunctional 27-kd myristylated protein produced by an ORF located at the 3' end of the primate lentiviruses. Other forms of Nef are known, including nonmyristylated variants. Nef is predominantly cytoplasmic and associated with the plasma membrane via the myristyl residue linked to the conserved second amino acid (Gly). Nef has also been identified in the nucleus and found associated with the cytoskeleton in some experiments. One of the first HIV proteins to be produced in infected cells, it is the most immunogenic of the accessory proteins. The nef genes of HIV and SIV are dispensable *in vitro*, but are essential for efficient viral spread and disease progression *in vivo*. Nef is necessary for the maintenance of high virus loads and for the development of AIDS in macaques, and viruses with defective Nef have been detected in some HIV-1 infected long term survivors. Nef downregulates CD4, the primary viral receptor, and MHC class I molecules, and these functions map to different parts of the protein. Nef interacts with components of host cell signal transduction and clathrin-dependent protein sorting pathways. It increases viral infectivity. Nef contains PxxP motifs that bind to SH3 domains of a subset of Src kinases and are required for the enhanced growth of HIV but not for the downregulation of CD4.
- VPX** A virion protein of 12 kD found only in HIV-2/SIVmac/SIVsm and not in HIV-1 or SIVagm. This accessory gene is a homolog of HIV-1 vpr, and HIV-2/SIV carry both vpr and vpx. Vpx function in relation to xpr is not fully elucidated; both are incorporated into virions at levels comparable to gag proteins through interactions with Gag p6. Vpx is necessary for efficient replication of SIV in PBMCs. Progression to AIDS and death in SIV-infected animals can occur in the absence of Vpr or Vpx. Double mutant virus lacking both vpr and vpx was attenuated,

Landmarks

whereas the single mutants were not, suggesting a redundancy in the function of Vpr and Vpx related to virus pathogenicity.

STRUCTURAL PROTEINS/VIRAL ENZYMES The products of gag, pol and env genes, which are essential components of the retroviral particle.

REGULATORY PROTEINS Tat and Rev proteins of HIV/SIV and Tax and Rex proteins of HTLVs. They modulate transcriptional and posttranscriptional steps of virus gene expression and are essential for virus propagation.

ACCESSORY OR AUXILIARY PROTEINS Additional virion and non-virion- associated proteins produced by HIV/SIV retroviruses: Vif, Vpr, Vpu, Vpx, Nef. Although the accessory proteins are in general not necessary for viral propagation in tissue culture, they have been conserved in the different isolates; this conservation and experimental observations suggest that their role *in vivo* is very important. Their functional importance continues to be elucidated.

COMPLEX RETROVIRUSES Retroviruses regulating their expression via viral factors and expressing additional proteins (regulatory and accessory) essential for their life cycle.