HIV Sequence Compendium 2003

Editors

Thomas Leitner
Los Alamos National Laboratory

Brian Foley
Los Alamos National Laboratory

Beatrice Hahn
University of Alabama

Preston Marx
ADARC

Francine McCutchan
Henry M. Jackson Foundation

John W. Mellors
University of Pittsburgh

Steven Wolinsky
Northwestern University

Bette Korber
Los Alamos National Laboratory

Project Officer
James Bradac
Division of AIDS
National Institute of Allergy and Infectious Diseases

Los Alamos Database and Analysis Staff
Werner Abfalterer, Charles Calef, Brian Gaschen,
Kristina Kommander, Dorothy Lang, Ming Zhang

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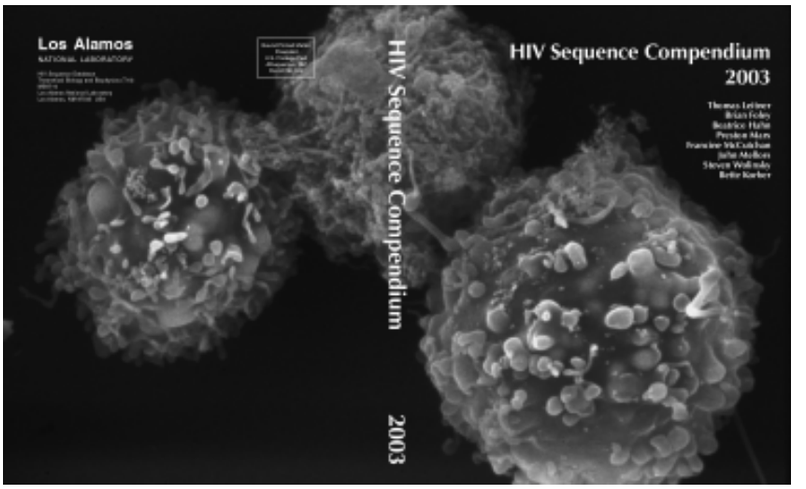
The Cover



This year's cover is a photo of HIV infected T lymphocytes, The projections from the cell surface are microvilli. The HIV particles are seen among the microvilli as small, barely visible, rounded dots. Large clusters of virus can also be seen. The photo was taken by Dr. h.c. Lennart Nilsson, Karolinska University Hospital, Stockholm, Sweden. Lennart Nilsson has develped new photgraphic methods and technical improvements including electron microscopy, opening new dimensions to scientific photography. His work on medical subjects have become know worldwide through publication such as "Behold Man" and "A Child is Born."

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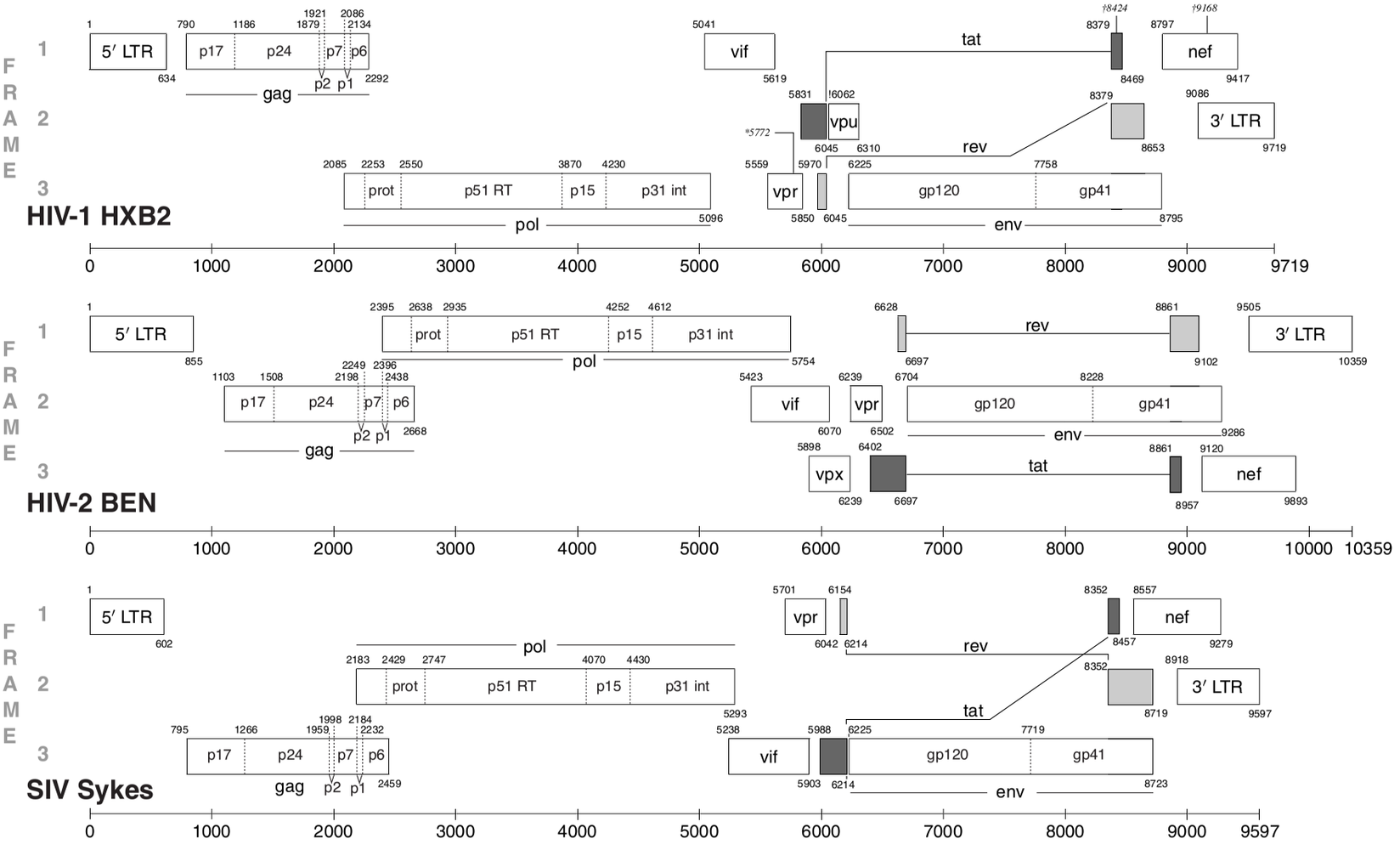
Introduction

This compendium is an annual printed summary of the data contained in the HIV sequence database. In these compendia we try to present a judicious selection of the data in such a way that it is of maximum utility to HIV researchers. In this years issue we have added a new section with HIV-2/SIVsmm complete genomes. Each of the alignments attempt to display the genetic variability within the different species, groups and subtypes of the virus.

At the time of publishing, there were 884 genomes longer than 7000 nucleotides available (and 907 sequences longer than 5000), and because of space limitations in the printed compendium we have omitted many sequences. These omissions were done considering redundant sequencing of certain isolates and patients as well as construction of phylogenetic trees with all available sequences. In section II, the HIV-1/SIVcpz complete alignment, we have included all subtypes and Circulating Recombinant Forms (CRFs), and thus the trend is to include at least the four reference sequences from each of the epidemiologically important virus variants. For some subtypes we have included more sequences to reflect their importance. In the future, if and when more subtypes and CRFs are described, there may be only space for the reference sequences. As always, tables with extensive background information gathered from the literature accompany the whole genome alignments. A more complete version of all alignments is available on our website, http://www.hiv.lanl.gov/content/hiv-db/ALIGN_CURRENT/ALIGN-INDEX.html .

Reprints of all reviews are available from our website in the form of both HTML and PDF files. As always, we are open to complaints and suggestions for improvement. With the effort that goes into producing these volumes, we sincerely hope they will be widely used by the research community. Inquiries and comments regarding the Compendium should be addressed to:

Dr. Thomas Leitner
Theoretical Division, T-10, MS K710, LANL, Los Alamos, NM 87545, USA
Ph: (505)-667-3898; fax: (505)-665-3493; e-mail: tkl@lanl.gov



Landmarks of the HIV-1, HIV-2, and SIV genomes. The gene start, indicated by the small number in the upper left corner of each rectangle normally records the position of the `a` in the `atg` start codon for that gene while the number in the lower right records the last position of the stop codon. For *pol*, the start is taken to be the first `t` in the sequence `ttttttag` which forms part of the stem loop that potentiates ribosomal slippage on the RNA and a resulting –1 frameshift and the translation of the gag-pol polyprotein. The *tat* and *rev* spliced exons are shown as shaded rectangles. In HXB2, **5772* marks position of frameshift in the *vpr* gene; !6062 indicates a defective `acg` start codon in *vpu*; *†8424*, and *†9168* mark premature stop codons in *tat* and *nef*. See Korber *et al.*, Numbering Positions in HIV Relative to HXB2CG, in *Human Retroviruses and AIDS, 1998* p. 102. Available from `http://www.hiv.lanl.gov/content/hiv-db/HTML/reviews/HXB2.html`

HIV/SIV PROTEINS

NAME	SIZE	FUNCTION	LOCALIZATION
Gag			
MA	p17	membrane anchoring; Env interaction; nuclear transport of viral core. (myristylated protein)	virion
CA	p24	core capsid	virion
NC	p7	nucleocapsid, binds RNA	virion
	p6	binds Vpr	virion
Pol			
Protease (PR)	p15	Gag/Pol cleavage and maturation	virion
Reverse transcriptase (RT), RNase H	p66 p51 p15	reverse transcription, RNase H activity	virion
Integrase (IN)	p31	DNA provirus integration	virion
Env	gp120/ gp41	external viral glycoproteins bind to CD4 and secondary receptors	plasma membrane, virion envelope
Tat	p16/p14	viral trancriptional transactivator	primarily in nucleolus/nucleus
Rev	p19	RNA transport, stability and utilization factor(phosphoprotein)	primarily in nucleolus/nucleus shuttling between nucleolus and cytoplasm
Vif	p23	promotes virion maturation and infectivity	cytoplasm (cytosol, membranes) virion
Vpr	p10–15	promotes nuclear localization of preintegration complex, inhibits cell division, arrests infected cells at G2/M	virion, nucleus (nuclear membrane?)
Vpu	p16	promotes extracellular release of viral particles;degrades CD4 in the ER;(phosphoproteinonly in HIV-1 and SIVcpz)	integral membrane protein
Nef	p25–p27	CD4 and class I downregulation (myristylated protein)	plasma membrane, cytoplasm(virion?)
Vpx	p12–16	Vpr homolog (not in HIV-1, only in HIV-2 and SIV)	virion (nucleus?)

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 (MAtrix), p24 (CApsid), p7 (NucleoCapsid), 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 near the 3' end 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-2 exon (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 SIVmnd. 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 Vpr 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, 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.