Meeting report

Retroviruses at a glance

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Gaining better knowledge and understanding of retroviral genomes, life cycles and interactions within host cell machinery is a major requirement for anti-retroviral therapies. The International Retroviruses 2000 conference assembled reports describing new discoveries on fundamental steps of retrovirus biology. We have focused our report on the areas which seemed to show the most recent developments: the fusion process, entry, assembly-budding-maturation, and also new breakthroughs looking at endogenous retroviruses.

Retroviral particles possess a lipid bilayer envelope derived from the plasma membrane of the host cell. Env glycoproteins are localised on the envelope of the retroviral particles (Figure 1). They are cleaved into two polypeptides: the surface subunit is exposed on the virion surface and the transmembrane subunit is anchored in the virion envelope. In the mature retroviral particles, Gag polyproteins are cleaved by the retroviral protease (PR) into three major proteins, matrix, capsid and nucleocapside. Capsid proteins form the viral core where the two plus strand genomic RNA molecules are localised. The virions also contain reverse transcriptase and integrase encoded by the *pol* gene.

The life cycle of a retrovirus starts with the specific binding of particles to a host cell membrane, via cellular receptor and viral surface protein interactions (Figure 2). Fusion of the viral envelope with the cellular plasma membrane permits the viral core to enter into the infected cell. Then, the genomic RNA is reverse transcribed into a double-stranded DNA molecule. After nuclear translocation, the genomic viral DNA is integrated in the cellular genomic DNA by the integrase and is then called provirus. The proviral DNA structure is composed of long terminal repeat (LTR) sequences located at each end of the integrated genome, surrounding *gag*, PR, *pol* and *env* coding genes. Proviral DNA transcription and translation are performed by the cellular machinery. After assembly of RNA and viral proteins, particles bud from the plasma membrane and are further matured by protease cleavage of Gag polyproteins.

The transmembrane subunit of the viral envelope protein was traditionally thought to contain all the domains required

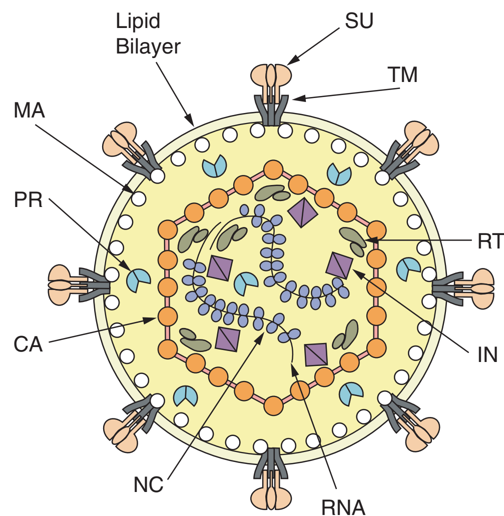

Figure 1
Schematic structure of a retroviral particle. TM, transmembrane components; SU, surface components; IN, integrase; CA, capsid protein; MA, matrix protein; NC, nucleocapsid protein; RT, reverse transcriptase; PR, retroviral protease. Adapted with permission from *Retroviruses*, Chapter 1, by PK Vogt, edited by JM Coffin, SH Hughes and HE Varmus, Cold Spring Harbor Laboratory Press, 1997.

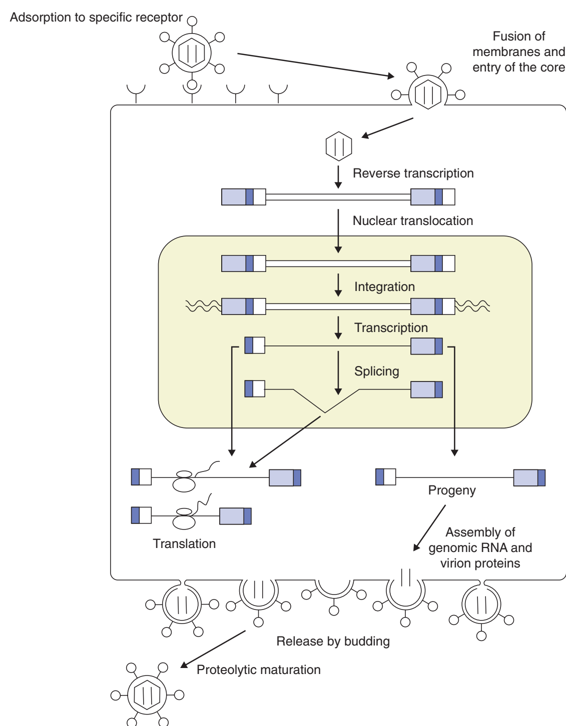

Figure 2
The retroviral life cycle. Adapted with permission from *Retroviruses*, edited by JM Coffin, SH Hughes and HE Varmus, Cold Spring Harbor Laboratory Press, 1997.

for membrane fusion during entry of retroviruses. In addition, to allow the viral and target cell membranes to get closer to each other, the binding subunits seem to be dissociated. Previous data have shown that changes in His8 of the surface binding subunit of ecotropic Moloney murine leukemia virus abolish entry and induction of syncitia, but have no effect on receptor binding. Lorraine Albritton (University of Tennessee, Memphis) showed that His8 is not required either for fusion peptide exposure, or for the fusion of the outer leaflet of the virion and cellular membranes, but is essential for pore formation. The fusion of the His8 mutant is arrested after the fusion of the outer leaflet of the virion envelope and the cellular membrane, but before the fusion of the inner lipid bilayers (hemifusion state). These results showed that His8 is part of the binding subunit domain that co-operates with the fusion peptide and transmembrane anchor in fusion pore formation. Moreover, these data also suggest that the binding subunit does not dissociate during the conformational changes that expose the fusion peptide, but remains as a critical part of the complex that catalyzes fusion pore formation.

In contrast to the recent progress in understanding receptor-mediated entry of HIV, little is known about HIV-cell interactions that occur after entry but early in infection. To study that particularly early step, Thomas Hope (Salk Institute for Biological Studies, La Jolla) labeled HIV cores by generation of a Vpr-green fluorescent protein (GFP) fusion protein. Observation of these labeled HIV particles in living cells after infection revealed that the particles moved in linear paths, suggesting possible interaction with cytoskeletal components. Co-localization analysis confirmed that the labeled particles are indeed associated with microtubules. When microtubules are stabilized by taxol treatment, which promotes microtubule polymerization, the labeled particles remained attached to the cytoskeleton after detergent extraction, whereas they were found in the soluble fraction in the absence of taxol treatment. These results suggested that HIV and microtubules interact and hence, as also seen in herpes virus and adenovirus, that HIV may use microtubules to move to the nucleus as a normal part of its life-cycle.

The assembly process of lentiviruses such as HIV-1 takes place at the plasma membrane. It is well established that this process is promoted by Gag proteins, but the specific mechanisms by which assembly occurs remain to be determined. Recent studies have suggested that the plasma membrane contains microdomains with distinctive protein and lipid compositions. One type of microdomain, or raft, is characterized by a high concentration of saturated lipids, such as sphingolipids and cholesterol. Some proteins become associated with the rafts, whereas some are excluded. Eric Freed (National Institute of Allergy and Infectious Diseases, NIH) reported that the use of agents disrupting raft structure severely impairs HIV-1 particle release from both HeLa and CEM cell lines without affecting general proteins synthesis or trafficking; a significant proportion of both cell- and virion-associated Gag was recovered in lipid rafts. Studies with carboxyl-terminus truncated Gag mutants reveals that strong Gag-Gag interactions are not required for efficient membrane transport, but are necessary for association with lipid rafts. Using an HIV-1 reporter virus encoding a protein localized in lipid rafts - the human alkaline phosphatase (PLAP) - Christopher Aiken (Vanderbilt University School of Medicine) showed that pr55Gag and PLAP co-localized in fractions of sucrose gradient purified virions. The presence of PLAP in purified virions is due to the presence of lipid rafts and suggests that HIV-1 assembly occurs at highly structured lipid rafts within the cells.

Late stages of viral assembly and release from the host cell are mediated by a particular region of the retroviral Gag proteins named L (for late) domain. L-domain(s) are located in different location within the Gag polyprotein among different viruses and are interchangeable from one virus to another; they possibly interact with a host cellular factor harboring a Trp-Trp motif that mediates protein-protein interactions as in many signaling and cytoskeletal proteins. Using several release-defective HIV-1 and Rous Sarcoma Virus (RSV) Gag minimal contructs, Heinrich Göttlinger (Dana-Farber Cancer Institute) reported that the presence of L-domains induces ubiquitination of

minimal Gag constructs and that residues essential for L–domain activity are also essential for ubiquitination, suggesting that L-domains recruit ubiquitin ligases, maybe to modify the membrane cytoskeleton in the area of the virus bud. The group of Lynn VerPlank (State University of New York at Stony Brook) used a different approach: using a two-hybrid system and co-immune precipitation assay, they observed that HIV Pr55Gag binds to Tsg101, a homolog of E2 ubiquitin conjugating enzyme. Deletion of the L-domain motif of HIV-1 (PTAPP, using the single letter code for amino acids) abolishes this binding. Finally, a competition assay showed that the PTAPP motif in HIV-1 is necessary but not sufficient for pr55Gag-Tsg101 interactions. By contrast, the L-domain motif of RSV (PPPPY) is sufficient for interaction with cellular binding partners. This suggests that HIV and RSV might use a common pathway for budding, but involve two different cellular proteins associated with ubiquitination.

After budding, the particles are immature, as Gag polyproteins are still uncleaved. The particles are mature and then infectious only when Gag polyproteins are cleaved by the retroviral protease. The mature, infectious HIV retroviral particle contains a conical capsid (CA) composed of about 1500 CA protein subunits, which organises the RNA genome for uncoating and replication in a new host cell. Thus, CA plays an essential role in the HIV-1 life cycle. Crystal structures show that the CA molecule is shaped by seven helices in the amino-terminal domain, and four helices in the carboxy-terminal domain. *In vitro*, HIV-1 capsid spontaneously assembles into helical tubes and cones. Electron cryomicroscopy and image reconstruction have shown that those helical tubes are composed of hexameric rings. CA hexamers are linked to six neighboring hexamers via their carboxy-terminal domains. To test the structural model and to determine sites of the CA that are crucial for viral function, Wesley Sunquist (University of Utah) introduced 43 alanine mutations which scan all the surface of the CA without modifying the folding of the protein, and mutants were tested for distinct steps of the retroviral life cycle. Three mutations at the base of the CA carboxy-terminal domain severely diminished particle formation and lead to enlarged and oddly shaped particles. Four mutants in the amino-terminal domain located underneath the cyclophilin-binding loop implicated that this domain is also involved in retroviral assembly. Residues crucial for maturation and capsid assembly were clustered in helices 1 and 2. Finally, five mutants assembled normally but did not replicate, suggesting that the CA protein also has novel, non-structural functions.

Endogenous retroviruses (ERVs) are retroviral sequences that are integrated into each cell of all individuals belonging to the same species. ERVs are not silent genomic components, as they are expressed at the RNA and protein levels. The most characterized human endogenous retroviral (HERV) family is HERV-K. HERV-K proviruses are present in the genomes of humans, apes and old world monkeys, having first entered the primate genome after catarrhines diverged from platyrrhines about 40 million years ago. Approximately 50 full-length HERV-K proviruses are estimated to be present in the human germ-line. All human HERV-K proviruses described to date have mutations that are lethal for viral replication, but, some full-length open reading frames (ORFs) encoding the viral primary translation products, Gag-Pro-Pol, Env, and cORF/K-Rev are present in multiple individual proviruses, suggesting that HERV-K might be capable of replicating by complementation and also raising the possibility of recombination among co-packaged HERV-K genomes. Although HERV-K RNA and proteins are expressed in some tissue cell lines, however, HERV-K virions are not infectious. By searching for genetic polymorphism at HERV-K LTRs loci, Jack Lenz (Albert Einstein College of Medicine, New York) showed that the full-length provirus sequences that have been characterized to date in the human genome can be placed into three groups: one group corresponds to the common ancestor of gorilla, bonobos, chimpanzee and human; one group formed sometime after the divergence of Pan and Homo lineages; and one group formed during a period just prior to the emergence of modern *Homo sapiens*. Some of these proviruses have been duplicated with their cellular flanking sequences and are now present on multiple chromosomes. Moreover, some solo LTRs are present in the genome of some individuals where there are complete proviruses in other individuals. A new HERV-K provirus has been identified, HERV-K108, that has full-length ORFs for all viral proteins, but a cysteine for tyrosine substitution in the conserved YXDD motif of reverse transcriptase (RT). A variant called HERV-K108B, however, found in a Sub-Saharian African individual, possesses a YXDD motif in the RT domain, but also has two other lethal mutations.

HERV-K expression seems to be associated with testicular germ-cell tumors. cORF, one of the HERV-K encoded proteins, has a Rev-like function as it stabilizes unspliced and incompletely spliced viral transcripts and enhances their nuclear export. Annette Boese (Universitaetsklinik, Homburg, Germany) showed that the expression of HERV-K cORF protein induces tumor formation in nude mice. Identification of cellular pathways influenced by this Rev-homologous protein was performed by screening of a human testis cDNA library using the yeast-two-hybrid system. The PLZF (promyelocytic leukaemia zinc finger) protein was identified as a cORF interaction partner. The human PLZF gene encodes a Krüppel-like zinc finger protein. Functions of PLZF including regulation of cell growth, differentiation and apoptosis have been characterized in the heamatopoietic compartment. There are some hints towards further functions of PLZF in spermatogenesis and testis development. These results suggested that functional interaction of cORF and PLZF may occur in spermatocyte precursors and influence their ongoing differentiation.

Although the main steps of the retroviruses life cycles are now well established, little is known about most early and late events of infection or of the interactions between virus and cellular components after virus entry. A better understanding of the mechanisms by which retroviruses replicate and interact with infected cells is critical both for the development of anti-retroviral therapies and for the improved production of retroviral vectors for gene therapy.