Retroviruses

---The name **retrovirus** comes from the enzyme, **reverse transcriptase**.

---Reverse transcriptase (RT) converts the RNA genome present in the virus particle into DNA.

---RT discovered in 1970.

---Baltimore, Dulbecco and Temin.
---Retroviruses are widespread and found in mammals, birds, fish, worms etc.
Human Retroviruses

---Human T-cell leukemia virus discovered in 1981.
  Adult T-cell leukemia (1%)
  HTLV-associated myelopathy / Tropical spastic paraparesis (1%)

---Human immunodeficiency virus discovered in 1983.
  AIDS (>99%)
  Dementia, neurological complications (30%)
## The Family Retroviridae

<table>
<thead>
<tr>
<th>Genus</th>
<th>Example</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha retroviruses</td>
<td>Avian leukemia virus</td>
<td>ALV</td>
</tr>
<tr>
<td>Beta retroviruses</td>
<td>Murine mammary tumor virus</td>
<td>MMTV</td>
</tr>
<tr>
<td></td>
<td>Simian retroviruses</td>
<td>SRV</td>
</tr>
<tr>
<td>Gamma retroviruses</td>
<td>Murine leukemia virus</td>
<td>MLV</td>
</tr>
<tr>
<td>Delta retroviruses</td>
<td>Human T-cell leukemia virus</td>
<td>HTLV</td>
</tr>
<tr>
<td>Epsilon retroviruses</td>
<td>Walleye dermal sarcoma virus</td>
<td></td>
</tr>
<tr>
<td>Lentiviruses</td>
<td>Human immunodeficiency virus</td>
<td>HIV</td>
</tr>
<tr>
<td>Spumaviruses</td>
<td>Simian foamy virus</td>
<td>SFV</td>
</tr>
</tbody>
</table>
Retrovirus Morphology

From “Retroviruses” CSHL Coffin, Hughes, Varmus

A. Lenti HIV
B. Beta MMTV
C. Alpha ALV
Simple Retroviruses

e.g. murine leukemia virus (MLV)
Complex Retroviruses

```
gag-pol-env-tat-rev-nef-vif-vpr-vpu        HIV-1


gag-pol-env-tax-rex-HBZ                    HTLV
```

*Complex retroviruses* have additional genes that are described as regulatory or accessory.
Structure of retrovirus particles

- **membrane**
- **capsid CA**
- **integrase IN**
- **nucleocapsid NC**
- **TM**
- **SU**
- **ENV**
- **tRNA**
- **reverse transcriptase RT**
- **protease PR**
- **integrase IN**
- **RNA genome**
- **matrix MA**
- **nucleocapsid NC**
- **GAG**
RNA genome

Two copies of viral RNA are in each virus particle.

5'-Cap-R-U5---PBS--DLS--Ψ----GAG-POL-ENV------U3-R-AAAAAA-3'

Cap and poly A

R repeats

U5, U3 unique regions at each end

PBS primer binding site

Ψ(Psi) packaging signal

DLS dimerization site
1. Virions bind receptors on cell surface

2. Virion and cell membranes fuse and virus infects cell

3. Uncoating

4. Reverse transcription to form viral DNA

5. Integration into chromosome

6. mRNAs

7. Proteins

8. Virus particles bud from cell membranes

9. Virus particles mature and become infectious

Reverse transcriptase complex, RTC

Pre-integration complex, PIC

Integrase

Proviral DNA

Long terminal repeat (LTR)
The repeats and unique U5, U3 regions at the ends of the viral RNA become duplicated during reverse transcription to form the LONG TERMINAL REPEATS or LTRs.
Retrovirus integration sites

1. MLV near transcription starts, CpG islands

2. HIV along transcriptionally active genes

This difference is due to host proteins exploited by the PIC to dock onto chromosomes. HIV integrase in the PIC binds LEDGF.
Retroviral mRNA synthesis uses the integrated provirus as template.

HIV-1 LTR region/promoter
Simple retrovirus make two mRNAs
Translation of Gag and Gag/Pol precursor proteins

1. Gag is translated as a long precursor protein.

2. 5% of Gags are made as a Gag/Pol precursor.

3. Translational readthrough e.g. MLV

4. Frame shift e.g. HIV

Proteins produced: Gag
                Gag/Pol
Retroviral replication cycle
-budding of virions
Viral protease cleaves the Gag and Gag/Pol precursor proteins into mature MA, CA, NC Gag proteins and RT, IN, PR enzymes.

**Virion maturation** is essential for the virion to be infectious.
Transformation of cells by retroviruses

---Some retroviruses transform cells and cause tumors at high frequency in animals including cats, birds, mice and monkeys.

---These retroviruses carry an oncogene (v-onc).
Oncogenes

---*v-onc* genes are mutated cellular genes that have been transduced by retroviruses.

---The viral oncogene is part of the viral genome.

---The non-transforming cellular counterpart is known as a *c-onc*.

---The retrovirus delivers the transforming oncogene into the host cell's chromosomes via integration.
Transforming retroviruses

Replication competent

Rous sarcoma virus

Replication defective

Helper virus

v-ONC carrying genome
Production of oncogenic virus particles from a replication defective oncornavirus
## Examples of oncogenes

<table>
<thead>
<tr>
<th>Oncogene function</th>
<th>Example of v-oncogene</th>
<th>cell gene</th>
<th>Retrovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factor</td>
<td>sis</td>
<td>PDGF</td>
<td>Simian sarcoma virus</td>
</tr>
<tr>
<td>Tyrosine kinase receptors</td>
<td>erbB</td>
<td>EGFR</td>
<td>Avian erythroblastosis virus</td>
</tr>
<tr>
<td>G proteins</td>
<td>H-ras</td>
<td>ras</td>
<td>Harvey murine sarcoma virus</td>
</tr>
<tr>
<td>Non-receptor tyrosine</td>
<td>v-src</td>
<td>c-src</td>
<td>Rous sarcoma virus</td>
</tr>
<tr>
<td>kinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine/threonine kinases</td>
<td>v-mos</td>
<td>c-mos</td>
<td>Moloney murine sarcoma virus</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>v-myc</td>
<td>c-myc</td>
<td>avian myeloblastosis virus</td>
</tr>
</tbody>
</table>
Non acute transformation

---Retroviruses without an oncogene, still induce tumors, at a lower frequency.

---Transformation is caused by *insertional mutagenesis*.

---Integration of a provirus into a chromosome deregulates genes in close proximity.
Mechanisms of insertional mutagenesis

1. Promoter insertion.

1. **Enhancer insertion.** The LTRs carry enhancer regions, which can activate genes when inserted in the vicinity.
Retroviral vectors

---Aim to deliver a retrovirus-based genome that will integrate into the host cell chromosome and express only the gene of choice.

---Retroviral vectors are produced in "so-called" packaging cell lines.
Making a retroviral vector
Packaging a genome that encodes a therapeutically useful gene

Packaging signal  
host chromosome  
RNA genomes carrying therapeutic gene

Making a retroviral vector
Packaging a genome that encodes a therapeutically useful gene

Infection of target cells

Therapeutic gene
mRNA/protein

Packaging particles--
Carry RNA encoding the therapeutic gene

LTR
therapeutic gene
LTR

env mRNAs

Proteins

gag-pol mRNA

Proteins

env

gag-pol

LTR
# Envelopes for retroviral vectors

<table>
<thead>
<tr>
<th>Envelope</th>
<th>Infection of human cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV envelopes</td>
<td>amphotropic</td>
</tr>
<tr>
<td>Vesicular stomatitis virus</td>
<td>G protein</td>
</tr>
<tr>
<td>Ebola</td>
<td>GP protein</td>
</tr>
</tbody>
</table>
Retroviral vectors and dividing cells

Simple retroviruses e.g. MLV, cannot infect non-dividing differentiated cells.

Retroviral vectors based on HIV and other lentiviruses can infect non-dividing differentiated cells e.g. macrophages.
X-linked severe combined immunodeficiency
SCID-XL

1. SCID-XL results from a lesion in the gene for the common \( \gamma_c \) chain that is a subunit for several interleukin receptors e.g. IL-2R, IL-4R, IL-7R.

2. Children with SCID-XL do not form a mature immune system and must be isolated.
The IL-2 receptor

IL-2Rα | IL-2Rβ | γc
Correction of SCID-XL by retroviral gene therapy

---19 SCID-XL children were treated using retroviral gene therapy in two trials.

---A Murine Leukemia Virus (MLV) based retroviral vector carrying a functional IL-2R-γc gene to replace the defective version.

---For 17 of the children, the γc gene was successfully introduced and immune system restored.
Introduction of the $\gamma_c$ gene into bone marrow hematopoietic cells

Infection of BM hematopoietic stem cells

Viral RNA construct carrying $\gamma_c$ gene

Virions carrying RNA encoding $\gamma_c$

Immune reconstitution
Five children developed T-cell leukemia.

---The leukemic cells originated from re-infused T-cells.

---In four cases, the retroviral vector had integrated close to and upregulated the LMO2 gene.

---Upregulation of LMO2 is a contributor to the leukemias.
Safer vectors

• Lentivirus-based vectors.

• Modification of the LTR regions so that vector does not carry enhancer sequences.

• New gene editing approaches.
Human T-cell leukemia viruses

HTLV-I
HTLV-II

HTLV-3
HTLV-4
HTLV is a complex Retroviruses

Complex retroviruses have additional genes that are described as regulatory or accessory.
--- **tax** transactivates transcription.

--- Acts on HTLV LTR, stimulating transcription.

--- Binds GC rich regions on the LTR adjacent to CRE sites. Then interacts with CREB transcription factors bound to proximal CRE sites.

--- However, effects on other transcription factors are important e.g. NFκB.

--- Activates NFκB by stimulating dissociation with IκB
Rex regulates mRNA splicing

--- early in infection, multiply spliced mRNAs encoding tax and rex are produced.

--- When there is enough rex, it regulates splicing of viral mRNAs.

--- It binds RRE, a secondary RNA structure in the env RNA

--- Rex directs nuclear export of mRNAs before they are fully spliced. These mRNAs encode Gag/Pol and Env
HTLV-I and disease

About 20 million people worldwide are infected.

About 2-3% of infected individuals will suffer an HTLV-I related disease in their lifetime.

---Adult T-cell leukemia.

---HTLV associated myelopathy (HAM)/tropical spastic paraparesis (TSP).
HTLV-I epidemiology

HTLV-I is prevalent in south western Japan, the Caribbean and Central Africa.

In Japan: up to 35% in parts of Okinawa, 8-10% in Kyushu Province, 0-1.2% in non-endemic areas.

Worldwide: Japan, 8%; Uganda, 8%; Ghana, 9%; Jamaica, up to 6%; Dominican Republic, 2%; USA, 0.003-0.04%.

New York IVDA: prevalence of 9%.
Adult T-cell leukemia (ATL)

---About 1% of HTLV-I+ individuals eventually suffer adult T-cell leukemia (ATL).

---CD4+ T-cell tumor

---ATL takes years to develop.

---Thus further additional changes must be required for a malignant T-cell leukemia to develop.
Adult T-cell leukemia (ATL)

1. Tumor cells carry an integrated HTLV-I provirus.

2. In different tumors HTLV-I proviral DNA is integrated at different sites.

3. The integration site is NOT specific for ATL.

4. The mechanism of tumor formation is NOT due to insertional mutagenesis.

5. Neither does HTLV carry an oncogene.
The role of Tax in transformation and ATL

1. Tax activates NF$_{κB}$.

2. Host cell genes are upregulated or repressed.

3. Upregulated genes e.g. $IL-2$, $IL-2R\alpha$, $c-myc$.

4. Repressed genes include tumor suppressor, p53.

5. *These effects explain the capacity of tax alone to transform T-cells in vitro.*
The role of HBZ

1. Tax not always expressed in tumor cells.

2. A new gene identified: HBZ.

3. HBZ is expressed in tumor cells and like tax also induces cell proliferation.
HTLV-II

---Much rarer than HTLV-I

---In Amerindian populations of America and intravenous drug abusers

---Rare cases of hairy cell leukemias

---HTLV-associated myelopathy
## Summary of Retroviral Transformation

<table>
<thead>
<tr>
<th>Transformation via</th>
<th>Active in humans</th>
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<tbody>
<tr>
<td>Oncogenes</td>
<td>No</td>
</tr>
<tr>
<td>Insertional mutagenesis</td>
<td>Only in a gene therapy trial</td>
</tr>
<tr>
<td>HTLV-I tax (HBZ?)</td>
<td>Yes</td>
</tr>
</tbody>
</table>