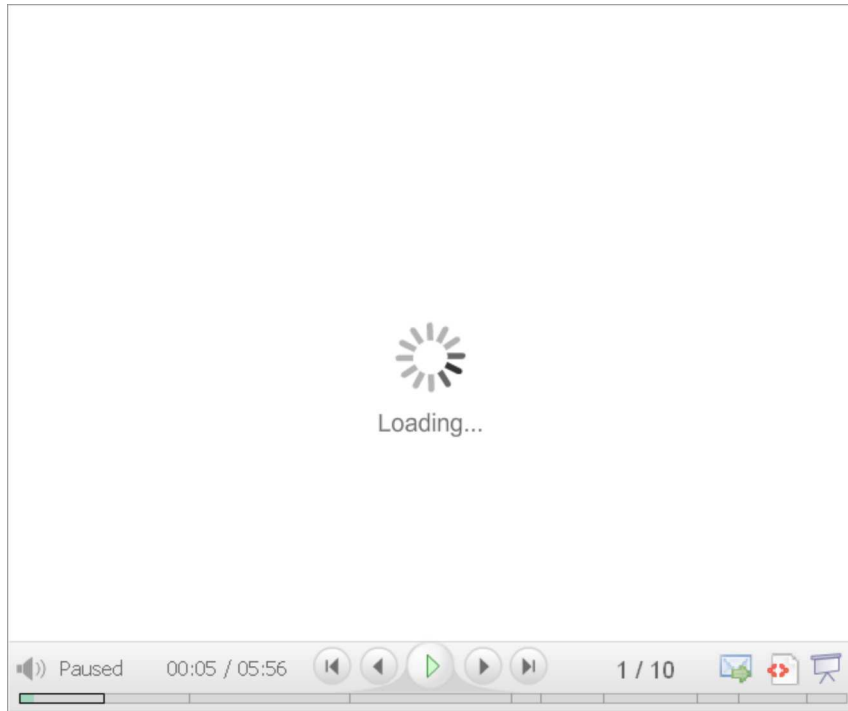


Retroviruses

[Retroviruses](#)



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Introduction:

Retroviruses have received much attention in recent years (even before the discovery of the first human retrovirus in 1981), but they have a long history:

- **1908:** Ellerman and Bang, searching for an infectious cause (bacterium) for leukaemia, studied leukaemia in chickens and succeed in transferring the disease from one to another by cell-free tissue filtrates (Ellerman, C., and O. Bang. Centralbl. Bakteriol. 46: 595–609).
- **1909:** Paul Ehrlich proposed his theory of 'immune surveillance' (Ehrlich, P. Über den jetzigen stand der karzinomforschung. Ned. Tijdschr. Geneesk. 5, 273–290):
 - Tumour cells frequently emerge in the organism
 - They are rapidly eliminated by the immune system
- **1910/1:** Peyton Rous transmitted solid tumours of chickens by transplanting tissue, but also isolated the infectious agent (Rous Sarcoma Virus: Rous, P. J. Exp. Med. 12:696–705; Rous, P. J. Exp. Med. 13:397–411).
This discovery was followed by many other examples of acutely transforming retroviruses, together with the structural characterization of the viruses involved.
- **1960's:** Howard Temin knew that retrovirus genomes were composed of RNA and observed that replication was inhibited by actinomycin D (inhibits DNA synthesis therefore he proposed the concept of reverse transcription (Nobel prize awarded to Baltimore and Temin, 1975).
- **1969:** Huebner and Todaro proposed the viral oncogene hypothesis - the transmission of viral and oncogenic information as genetic elements (rather than as a pathogenic response to a virus) - explains the vertical (germ line) transmission of 'cancers', first observed by Gross, 1951.
- **1970s:** Richard Nixon's 'war on cancer' (post Kennedy space programme - the race to the moon) - failed to find any retroviral agents which cause human cancer (many false alarms - but did pump a lot of money into biomedical research).
- **1981:** Human T-cell leukaemia virus discovered, the first pathogenic human retrovirus.
- **1983:** Human immunodeficiency virus discovered.

Most of the retroviruses we currently know (many!) infect vertebrates, but as a group, they have been identified in virtually all organisms including invertebrates - an evolutionarily successful design!

Taxonomy:

Group VI: RNA Reverse Transcribing Viruses			
Family	Genus	Type Species	Hosts
<i>Retroviridae</i>	<i>Alpharetrovirus</i>	<i>Avian leukosis virus</i>	Vertebrates

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	<i>Betaretrovirus</i>	<i>Mouse mammary tumor virus</i>	Vertebrates
	<i>Gammaretrovirus</i>	<i>Murine leukemia virus</i>	Vertebrates
	<i>Deltaretrovirus</i>	<i>Bovine leukemia virus</i>	Vertebrates
	<i>Epsilonretrovirus</i>	<i>Walley dermal sarcoma virus</i>	Vertebrates
	<i>Lentivirus</i>	<i>Human immunodeficiency virus 1</i>	Vertebrates
	<i>Spumavirus</i>	<i>Chimpanzee foamy virus</i>	Vertebrates
<i>Metaviridae</i>	<i>Metavirus</i>	<i>Saccharomyces cerevisiae Ty3 virus</i>	Fungi
	<i>Errantivirus</i>	<i>Drosophila melanogaster gypsy virus</i>	Invertebrates
<i>Pseudoviridae</i>	<i>Pseudovirus</i>	<i>Saccharomyces cerevisiae Ty1 virus</i>	Invertebrates
	<i>Hemivirus</i>	<i>Drosophila melanogaster copia virus</i>	Invertebrates

Historically, retroviruses were divided into groups based on their morphology in negatively-stained E.M. pictures:

- **A-type:** Also known as 'intracisternal particles'. Non-enveloped, (non-infectious???) immature particles only seen inside cells, believed to result from endogenous retrovirus-like genetic elements.
- **B-type:** Enveloped, extracellular particles with a condensed, acentric core and prominent envelope spikes, e.g. MMTV.
- **C-type:** As B-type, but with a central core and barely visible spikes - e.g. most mammalian and avian retroviruses (MLV, ALV, HTLV, HIV).
- **D-type:** Usually slightly larger (to 120nm) and spikes less prominent, e.g. MPMV.

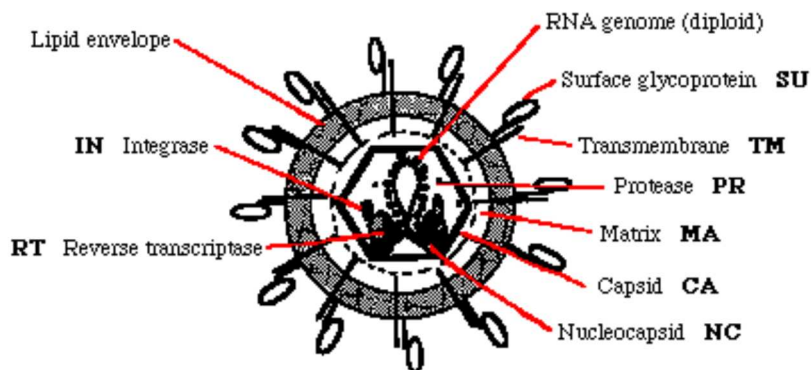
By and large, molecular genetic studies have borne out these morphologic differences, but have also largely replaced them - most comparisons now made on the basis of sequence conservation.

Retrovirus Structure:

There is considerable diversity between various types of retrovirus; the following is a generalized description of the particle. There is a universal nomenclature for retrovirus proteins:

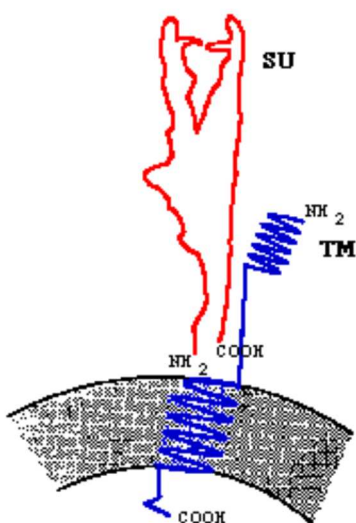
Name:	Protein:	Function:
MA	Matrix	matrix protein (gag gene); lines envelope
CA	Capsid	capsid protein (gag gene); protects the core; most abundant protein in virus particle
NC	Nucleocapsid	capsid protein (gag gene); protects the genome; forms the core
PR	Protease	Essential for gag protein cleavage during maturation
RT	Reverse transcriptase	Reverse transcribes the RNA genome; also has RNaseH activity
IN	Integrase	Encoded by the pol gene; needed for integration of the provirus
SU	Surface glycoprotein	The outer envelope glycoprotein; major virus antigen
TM	Transmembrane protein	The inner component of the mature envelope glycoprotein

All the above proteins are essential for replication; some retroviruses also encode additional essential and non-essential proteins.



Retroviruses have enveloped particles, somewhat variable in size/shape but ~100nm diameter. The envelope carries a virus-encoded glycoprotein, which forms spikes in the membrane. There are certain structural/functional similarities between the envelope glycoprotein and the influenza haemagglutinin (N.B: NO SEQUENCE SIMILARITIES). The mature protein is cleaved into 2 polypeptides:

- the outer envelope glycoprotein (**SU**), the major antigen of the virus, responsible for receptor binding, linked by disulphide bonds to:
- the trans-membrane glycoprotein (**TM**), holds the SU protein in the envelope, responsible for membrane fusion



Inside the membrane is the **matrix (MA)** protein, rather amorphous. This largely obscures the **capsid (CA)**, which is *believed* to be icosahedral. CA is the most abundant protein in the particle (~33% total weight). Inside the capsid is the **core** = RNA genome+NC protein+RT+IN. This is usually a conical, electron-dense structure clearly visible in -ve stained E.M. pictures (matrix and capsid appear amorphous).

[Turner B.G., Summers M.F. \(1999\) Structural Biology of HIV. J.Mol.Biol. 285: 1-32.](#)

Genome:

All retrovirus genomes consist of two molecules of RNA, which are s/s, (+)sense and have 5' cap and 3' poly-(A) (equivalent to mRNA). These vary in size from ~8-11kb. Retrovirus genomes have 4 unique features:

which is the first part of the genome to be reverse transcribed, forming the 3' end of the provirus genome (below). **Primer Binding Site:** 18nt complementary to the 3' end of the specific tRNA primer used by the virus to begin reverse transcription. **Leader:** A relatively long (90-500nt) non-translated region downstream of the transcription start site and therefore present at the 5' end of all virus mRNAs. **Polypurine Tract:** A short (~10) run of A/G residues responsible for initiating (+)strand synthesis during reverse transcription. **U3:** A unique non-coding region of 200-1,200nt which forms the 5' end of the provirus after reverse transcription; contains the promoter elements responsible for transcription of the provirus.



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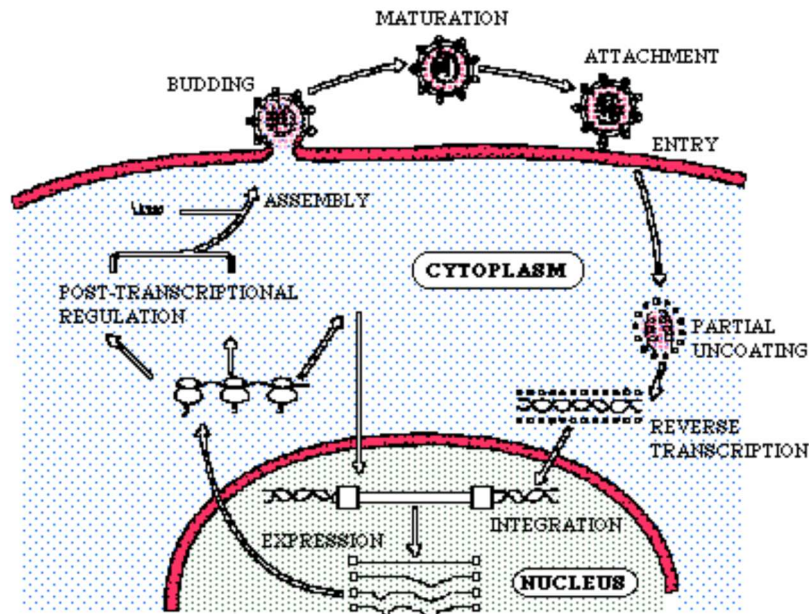


Replication:

To initiate the infection, the SU envelope glycoprotein binds to a specific receptor on the surface of the host target cell. The specificity of this interaction does much to determine the cell-tropism and pathogenesis of different retroviruses, or even different isolates of the same virus (e.g. HIV). Murine retroviruses (MLVs) are sub-divided on the basis of receptor-determined host species specificity:

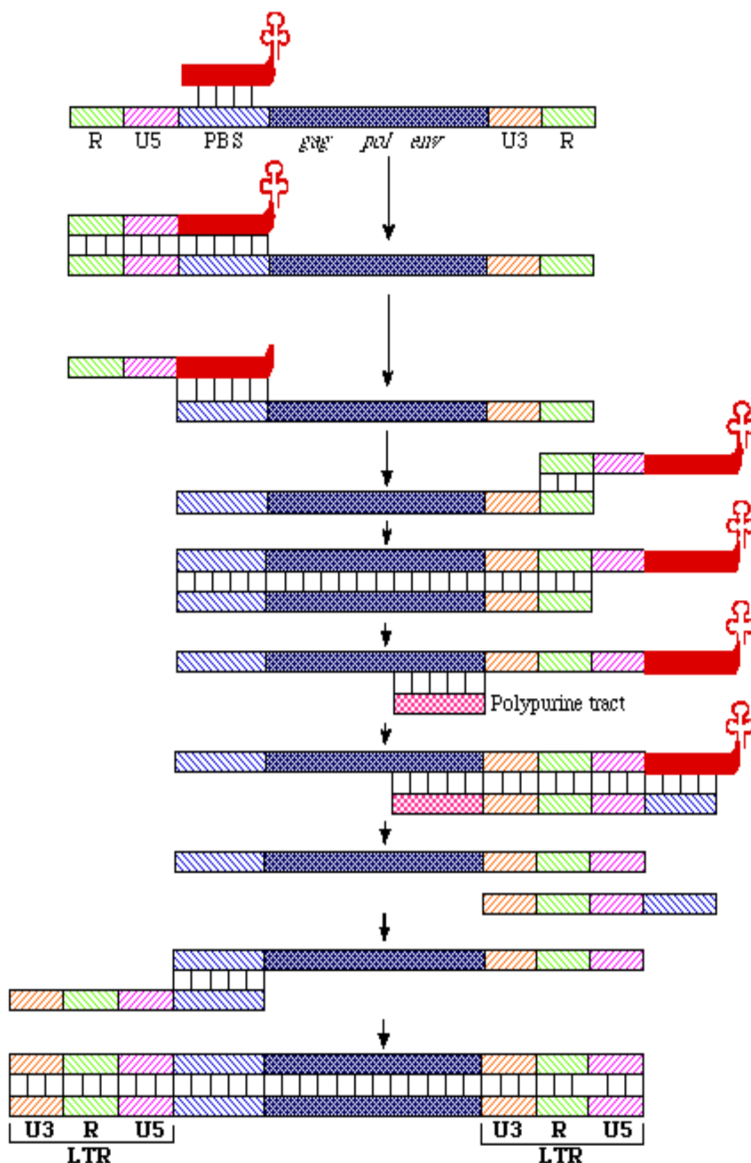
- **Ecotropic:** Infect only mouse cells.
- **Xenotropic:** Infect only non-mouse cells (e.g. rat, hamster).
- **Amphotropic:** Infect both mouse and non-mouse cells.

Interference between an exogenous virus and an endogenous virus of the same receptor specificity results in 'interference groups' of viruses (e.g. ALVs). In recent years, a number of different retrovirus receptor molecules have been identified: [Sommerfelt M.A. \(1999\) Retrovirus receptors. J.Gen.Virol. 80:3049-3064](#). See also: Restriction factors: a defense against retroviral infection [Bieniasz P.D. \(2003\) Trends in Microbiology 11: 286-291](#).



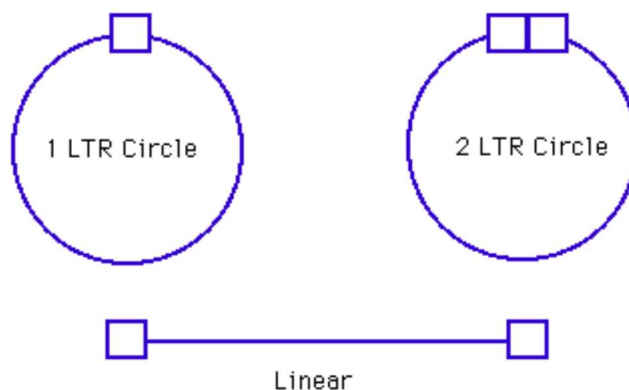
It is probable that receptor binding results in conformational changes in the glycoprotein spike, revealing the (previously masked) fusion domain in the TM protein and resulting in fusion of the virus envelope with the cell membrane. Penetration and uncoating are poorly understood, but it is now known that uncoating is only partial, resulting eventually in a core (nucleocapsid) particle within the cytoplasm. Reverse transcription occurs inside the ordered structure of this core particle - with the reactants (RT + RNA + nucleotides) free in solution, reverse transcription is initiated but cannot be completed, and aborts soon after.

Reverse Transcription:



Reverse Transcription: The Movie

The d/s DNA product formed by this reaction is known as the **provirus** (c.f. 'prophage') and differs from the vRNA in being longer by one U3,R,U5 sequence. As a result, there is a direct repeat of this sequence present at each end of the provirus genome, and these are known as the long terminal repeats (LTRs). Three forms of provirus DNA are found in all infected cells:

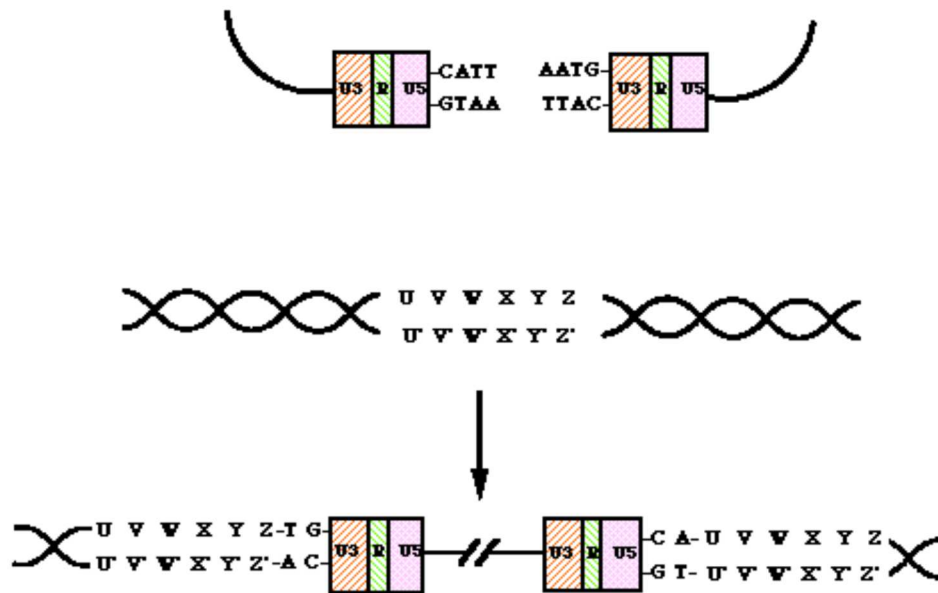


It is not clear how these are related to one another, but the circles probably form by intracellular

ligation. The linear and 2-LTR circle forms are infectious (unlike the (+)sense vRNA!). Reverse transcription occurs in the cytoplasm, after which the provirus DNA migrates into the nucleus.

Integration:

Catalysed by the IN polypeptide (part of the RTase complex). Integration is a highly specific reaction with respect to the provirus, but random with respect to host cell DNA. Formerly, it was thought that the 2-LTR circle was the substrate for integration, but it is now believed that the linear form (probably the direct product of reverse transcription) is the actual substrate used.



The ends of the LTRs consist of inverted repeats of 4-6 bp. These are brought together to form a cleavage site for IN and are cleaved to form a staggered cut. This molecule is then inserted into the host cell DNA. The net result of the integration process is that:

1. The integrated provirus contains 1 or 2 less bases at the end of each LTR
2. The ends of the integrated LTRs always have the same sequence: 5' - TG...CA - 3'
3. 4-6 bp of host cell DNA flanking the integrated provirus are duplicated.

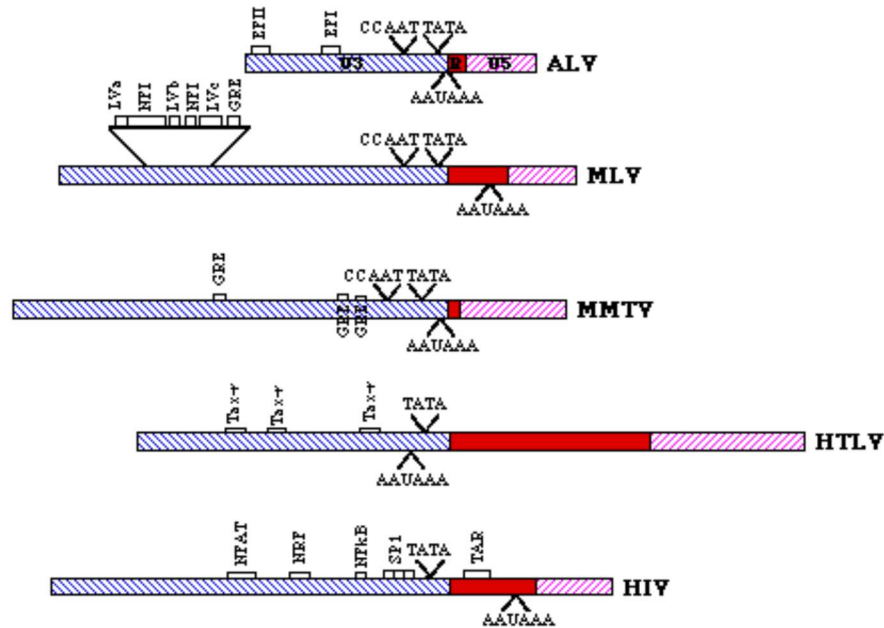
These observations can be explained by a model where a staggered cut (5' overhang) is introduced into both the ends of the LTRs and the host cell DNA, followed by joining of the cut ends and repair of the free 3' ends. Once integrated, the provirus is present for the lifetime of the cell (think about germ-line integration). There is no specific mechanism for excision of the provirus (c.f. lambda), and the infected cell cannot be 'cured'.

Gene expression:

Retroviruses use the cellular transcriptional machinery for expression (although a few encode additional transcriptional and post-transcriptional regulatory factors - HTLV and HIV). Therefore they are expressed like cellular genes. To compress maximal information into a small genome, they make use of a number of 'tricks', such as splicing and ribosomal frameshifting.

LTR Structure:

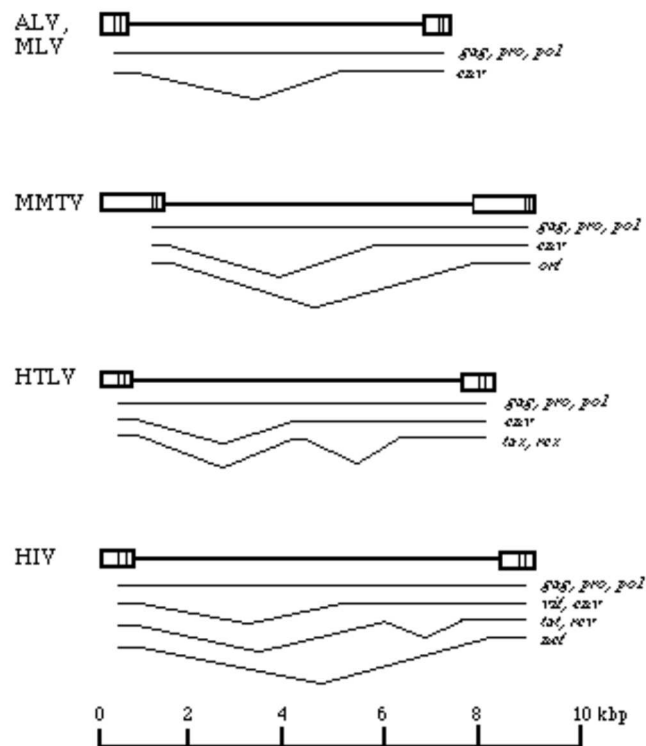
The U3 region of the LTR contains the promoter elements responsible for the initiation of transcription:



In recent years, various LTRs have been intensively studied and dissected by molecular techniques, including:

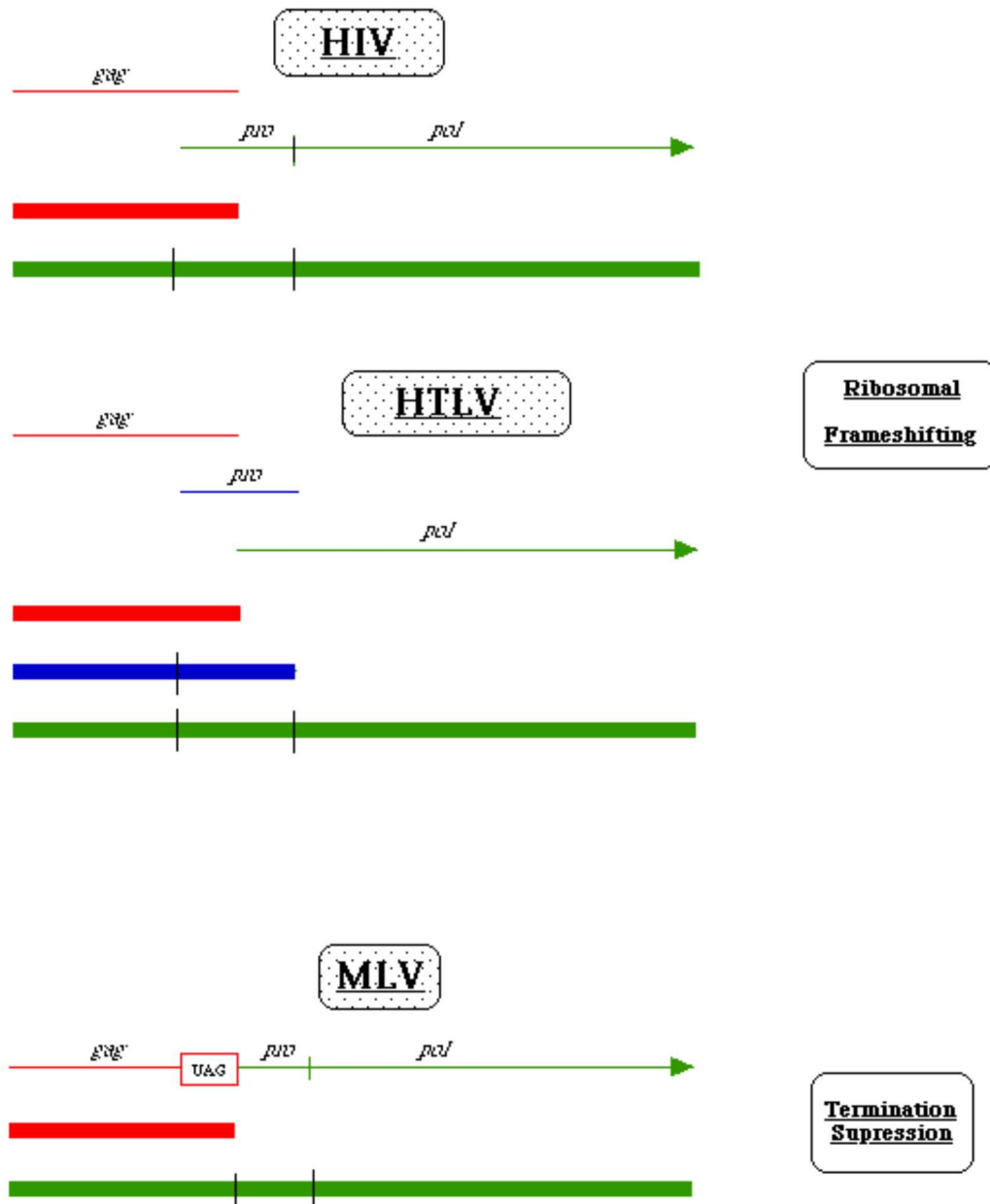
- nucleotide sequencing (and comparison with cellular promoter elements with known functions)
- nuclease protection studies:
 - S1 protection to determine precise transcription start sites
 - DNase I protection to determine DNA-binding protein sites
- in vitro transcription studies (reconstitute 'the nucleus' in vitro).

Splicing:



Splicing is regulated by the cellular apparatus which interacts with cis-acting sequences present in the mRNA. The proteins encoded by *gag*, *pol* and *pro* (see below) genes are expressed from a full length genomic RNA (= vRNA). The env protein is expressed from a spliced mRNA. In more complex retro's, e.g. HTLV, Lentiviruses, there are multiply spliced mRNAs are produced. Pattern of splicing in HIV is very complex!

Expression of the protease gene: *pro* overlaps *gag* and/or *pol*, but is still expressed from the same full-length mRNA. Different viruses have a variety of post-transcriptional strategies to do this:



Assembly:

- **B- & D-type viruses:** Capsid/nucleocapsid assembly occurs in the cytoplasm, later bud out through the cell membrane, acquiring the envelope during this process.
- **C-type viruses:** Assembly occurs at the cell surface. Thickened patches begin to form in the membrane (env proteins on outer surface, gag proteins inside).

The genome is packaged as the particle buds out through the membrane. With both types, **maturation** occurs after the particle has budded, by cleavage events catalysed by the protease. Considerable structural changes occur during this process, resulting in the smooth gag shell of the [immature particle](#) being completely rearranged and leading to the condensation of the core visible in [mature particles](#). N.B. - some types of retrovirus, notably Lentiviruses, are capable of infecting cells by direct cell-to-cell contact, without the formation of infectious extracellular particles.

[Freed EO. \(2004\) HIV-1 and the host cell: an intimate association. Trends Microbiol. 12: 170-177.](#)

[A negatively stained electron micrograph of HIV \(C-type\) particles.](#)

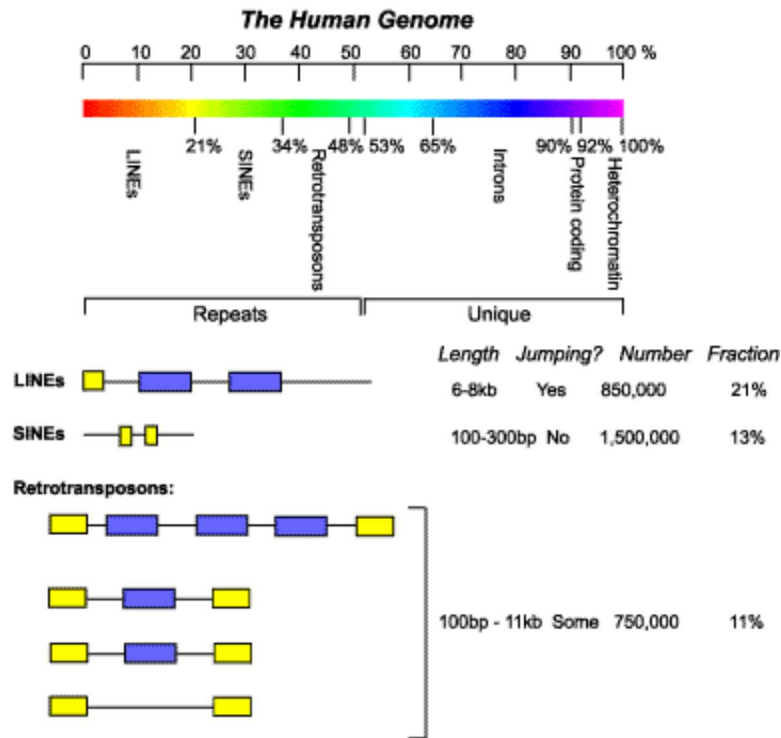


Retrovirus Genetics:

The genetics of retroviruses are complex:

- High mutation rate - reverse transcription is an error-prone process.
- Recombination - occurs during recombination, promoted by the combination of 2 strands of RNA into 1 d/s DNA provirus.
- Interactions with the host cell - insertional mutagenesis, transduction.

Retrotransposons - endogenous retrovirus-like genetic elements:



Much of the human genome consists of interspersed repetitive DNA sequences. The origin these sequences seems to have been retrotransposition in the germ line, generated by:

- transcription of DNA templates by RNA polymerase
- reverse transcription into DNA by reverse transcriptase
- insertion into new genomic locations, increasing the number of genomic copies of the sequence

~11% of the mammalian genome is composed of retrovirus-like **retrotransposons**: "transposable elements in which transposition involves a process of reverse transcription with an RNA intermediate similar to that of a retrovirus". Compare this with only ~2.5% of the human genome which encodes unique (non-repeated) genes!

Alu and L1 are the major families of human interspersed repeated DNA, amounting to 10-15% of the genome. Another type of repetitive DNA element consists of retrovirus-like elements (RLEs), or human endogenous retroviruses (**HERVs**), representing about 7% of the human genome. Their structure closely resembles that of retroviruses, carrying internal sequences with homology to *gag*, *pol*, and sometimes *env* open reading frames flanked by long terminal repeats. Similar sequences occur in all organisms, from yeast to vertebrates. [Bannert N, Kurth R. Retroelements and the human genome: new perspectives on an old relation. Proc Natl Acad Sci USA. 101: 14572-14579, 2004.](#)

[Phoenix from the ashes: The 5 million year old virus](#)

[Where do all these retroviruses come from?](#)

So what have retroviruses ever done for us?

[Mi, S. et al.\(2000\) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis Nature 403: 785-9.](#)

Pathogenesis:

Retroviral pathogenesis has concentrated on oncogenesis & more recently, AIDS, but retroviruses also cause a variety of haematopoietic and neurological conditions, including:

- Paralysis
- Wasting
- Ataxia
- Arthritis
- Dementia
- Neuropathy

It was recently reported that an ancient retrotransposon insertion is the cause of Fukuyama-type muscular dystrophy, one of the commonest autosomal recessive disorders in Japan ([Kobayashi, K. et al, \(1998\) Nature 394: 388-392](#)). To date this is the only known instance of insertional mutagenesis of the human genome caused by this type of element, but other examples look certain to be discovered in future.

[Retroviruses are under active development as vectors for gene therapy.](#)

[Pretend to be a Retrovirus!](#)

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The image is a screenshot of a Google News search page. At the top, there are navigation links for Web, Images, Videos, Maps, News, Shopping, Gmail, and more. A search bar contains the word "retrovirus" and a "Search" button. Below the search bar, the "Google news" logo is visible. The main content area is titled "News" and features a sidebar on the left with navigation options: "All news" (with an "Images" link), "Any recent news" (with links for "Past hour", "Past day", "Past week", "Past month", "1998-2007", "1991-1997", "1989-1990", and "1986-1988"). The main content area displays a news article titled "Musings on Retrovirus XMRV and EmpowHer (blog) - 1 hour ago". The article text reads: "The recent discovery of **retrovirus** XMRV surprising, this is very new research. ... [Link to virus found](#) UNR The Nevada Sage [all 3 news articles »](#) [Email this story](#)". Below the article text is a small photo of a man sitting on a couch. To the right of the photo is another article titled "Chronic Fatigue Syndrome Transmitted?" with the text "About - News & Issues (bl" and "Among the many questions".

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