

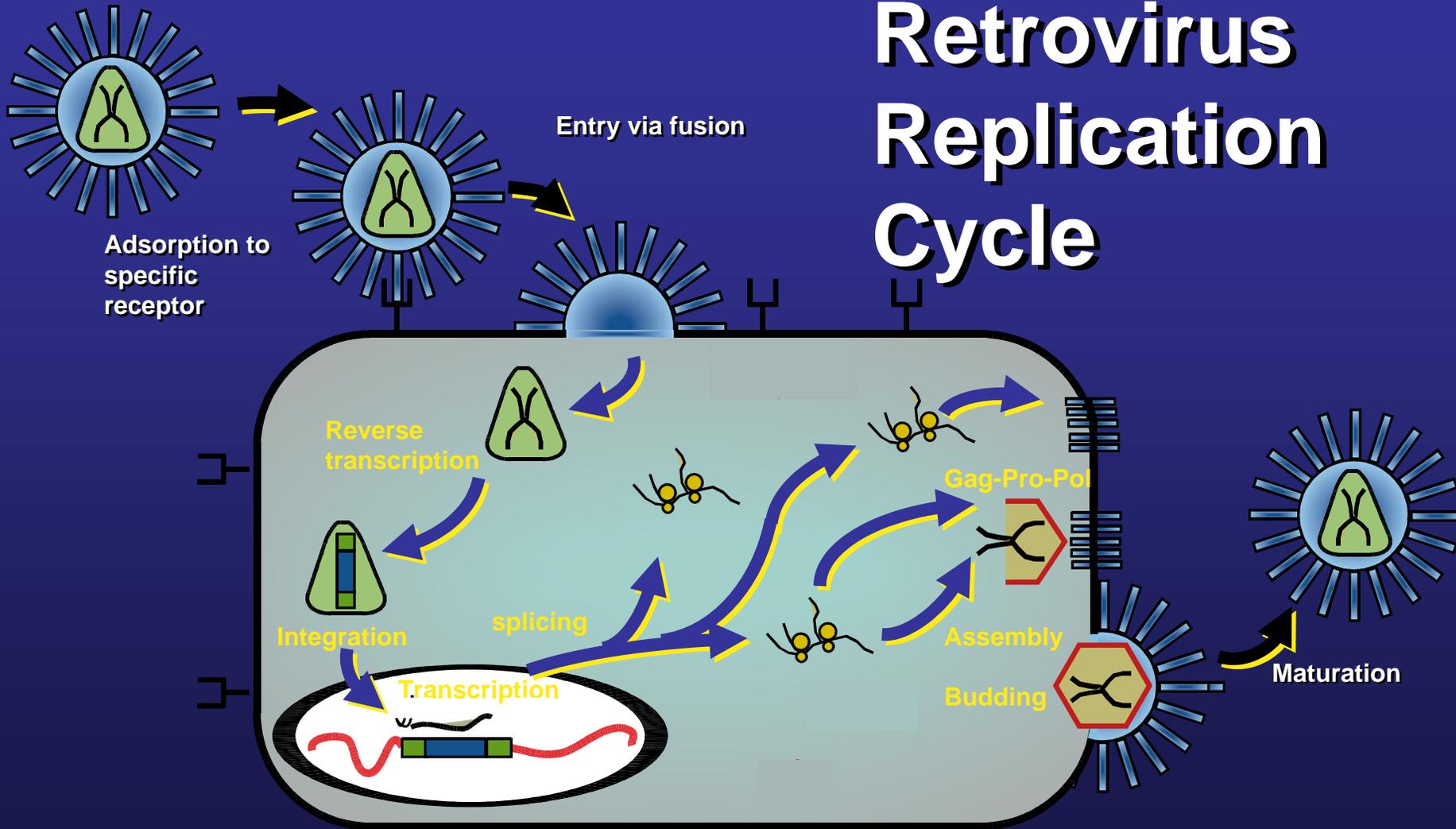
Significance of Retrovirus Contamination Of Cell Substrates

- 1. Properties of retroviruses**
- 2. Consequences of retrovirus contamination**
- 3. Sources of retrovirus contamination**
- 4. Assays for contaminating retroviruses**
- 5. Dealing with contaminating retroviruses**

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The Retrovirus Replication Cycle



Important Properties of Retroviruses

- 1. Require specific receptor for infection.**
 - Wide variety of receptors leads to great diversity in host and tissue specificity.
- 2. RNA genome; replicate via integrated DNA intermediate.**
 - Reverse transcriptase is diagnostic for virions.
 - Provides a mechanism for persistence and pathogenesis.
 - Infection of germ line can lead to endogenous proviruses.
- 3. Enveloped virions - formed by budding.**
 - Similar in physical and chemical properties to many other enveloped viruses.
- 4. Most do not kill or maim the infected cell.**

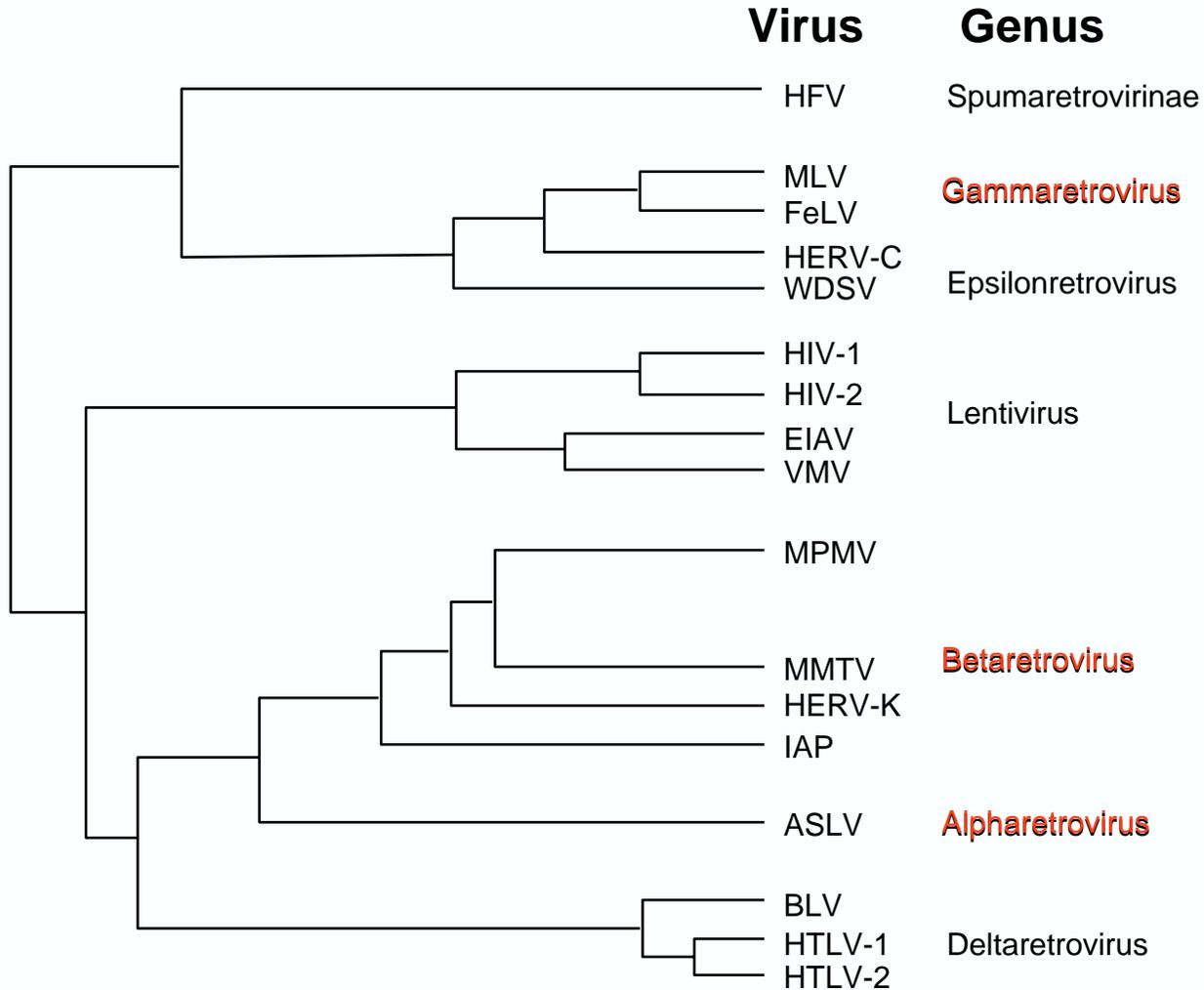
Exogenous Retroviruses

1. 2 general types: simple and complex.
2. Simple retroviruses:
 - Encode only virion proteins (and sometimes a few more).
 - Have endogenous relatives.
 - Usually transmitted vertically (mother to offspring).
 - Often incapable of infecting adults.
 - Include Alpha-, Beta, and Gammaretroviruses.
3. Complex retroviruses
 - Encode additional proteins including transactivators that regulate expression.
 - Have no known endogenous relatives.
 - Can be transmitted to immune competent adults.
 - Include Delta, Epsilon, Lenti, Spuma viruses.

Endogenous Retroviruses

1. Remnants of germ line infections by exogenous retroviruses.
2. Became fixed in the host species.
Some confer protection against future infections by the same or similar viruses.
3. Inherited like normal genes.
4. Present in every vertebrate and many invertebrates.
5. Expressed at a very low level due to CpG methylation, but can be induced by various means.
6. Comprise a large fraction of the host genome 6-8% in humans (More proviruses than genes).

The Retrovirus Family Tree



Alpharetroviruses

(Avian Type C Viruses)

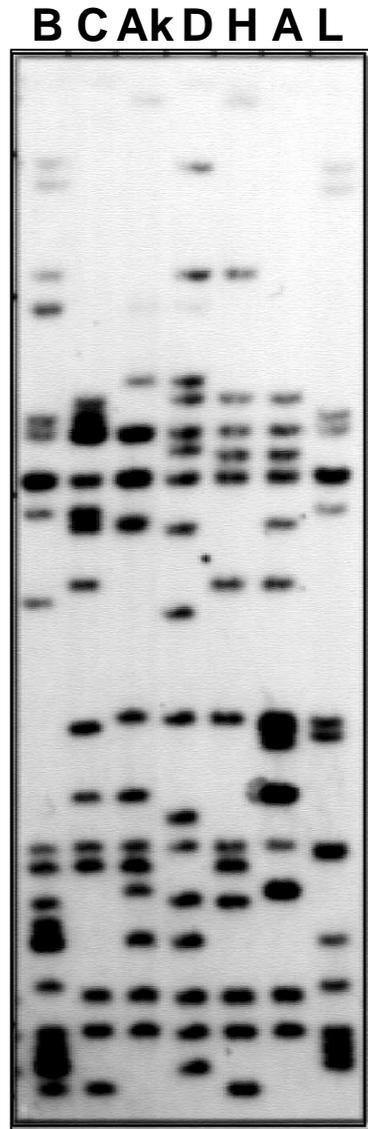
- 1. Simple retroviruses that include avian leukosis and sarcoma viruses.**
- 2. Cause a wide variety of diseases, including malignancies, wasting, and others in lab and natural settings.**
- 3. Most are transmitted vertically, from mother to offspring, and can infect only newborns.**
- 4. Exogenous ALV is common in commercial chicken flocks, and can cause serious problems.**
- 5. Have endogenous relatives in the genomes of many birds, both recent (0-10 or so proviruses per genome in chickens) and ancient (Ev-0).**
- 6. Viruses derived from endogenous proviruses are generally non-pathogenic and replicate slowly.**
- 7. Some endogenous proviruses (both ALV and Ev-0) can give rise to noninfectious, but RNA and RT positive, particles.**

Gammaretroviruses

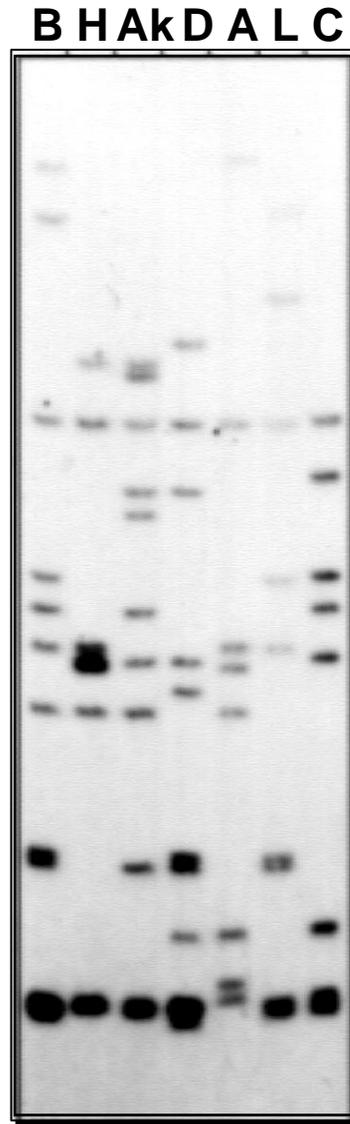
(Mammalian C-Type Viruses)

- 1. Simple retroviruses that include murine leukemia virus (MLV), feline leukemia virus (FeLV), gibbon ape leukemia virus (GALV), reticuloendotheliosis virus (REV), and many others.**
- 2. Cause a wide variety of diseases, including malignancies, immunodeficiencies, and others in lab and natural settings.**
- 3. Have endogenous relatives in the genomes of all mammals and many other vertebrates.**
- 4. Most are transmitted vertically, from mother to offspring, and can infect only newborns.**
- 5. Viruses derived from endogenous proviruses are generally non-pathogenic and replicate slowly, but some can become pathogenic by recombination (with related viruses) and mutation.**

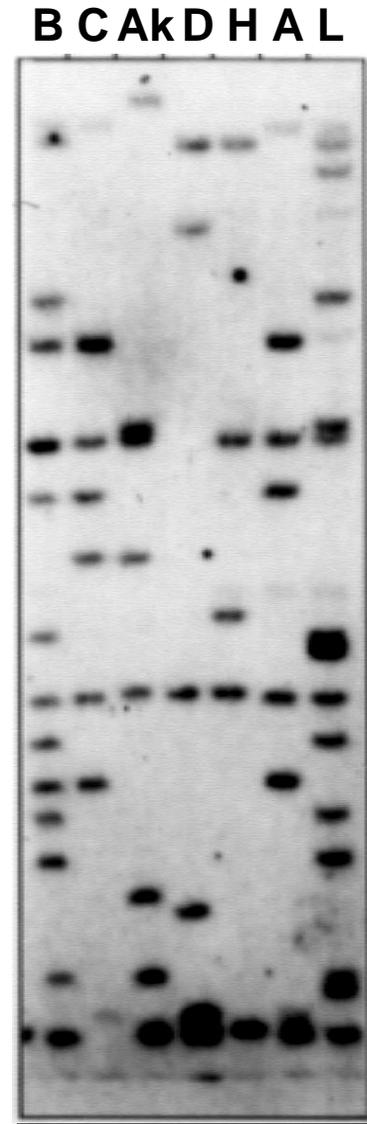
Distribution of Endogenous Proviruses in Inbred Mice



Pmv



Mpmv



Xmv

HERV-K

(Human Endogenous Viruses)

- 1. Simple retroviruses that infected our primate ancestors between < 1 and 30 million years ago**
- 2. Related to betaretroviruses like mouse mammary tumor virus and primate D type viruses.**
- 3. No infectious members are known, but a few have complete or nearly complete sequences with no obvious lesions.**
- 4. About 50 or so proviruses in each of our genomes.**
- 5. At least one provirus is expressed as visible particles in all human placenta, and some tumors (teratocarcinomas, breast cancer, and others).**
- 6. There is some variation in content among individuals.**

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Retroviral Pathogenesis

1. Wide variety of disease models

- Malignancy of many types
- Immunodeficiency
- Anemia
- Neurological
- Bone, joint, etc.

2. Varied Mechanism

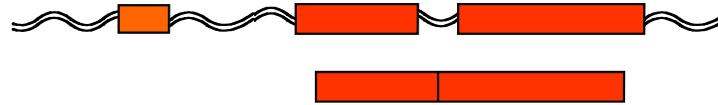
- Introduction or “activation” of oncogene
- Disruption of signaling
- Direct or immune-mediated cell killing

3. In general, requires massive infection resulting from extensive replication.

- Replication defective viruses unlikely to pose a significant hazard.

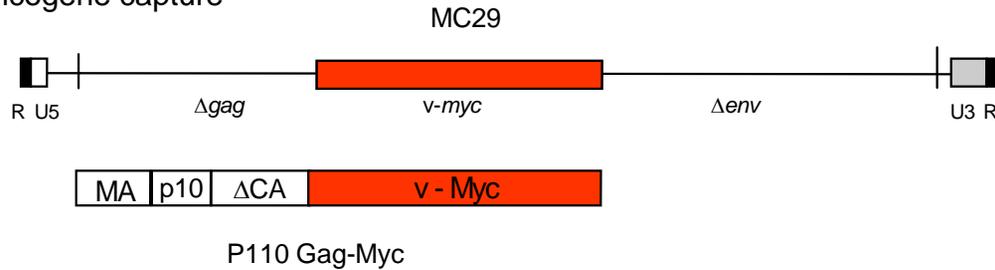
Mechanisms of Oncogene "Activation"

c-myc locus

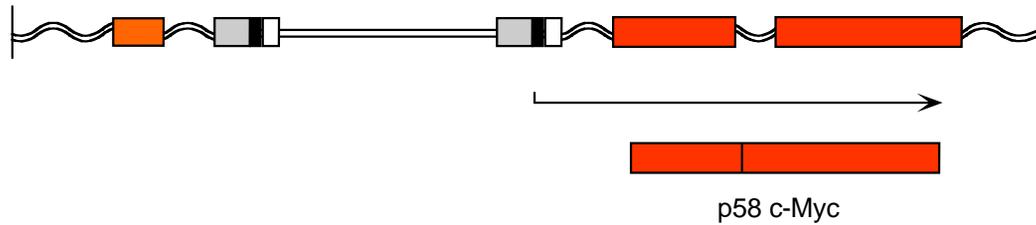


p58 c-Myc

A. Oncogene capture

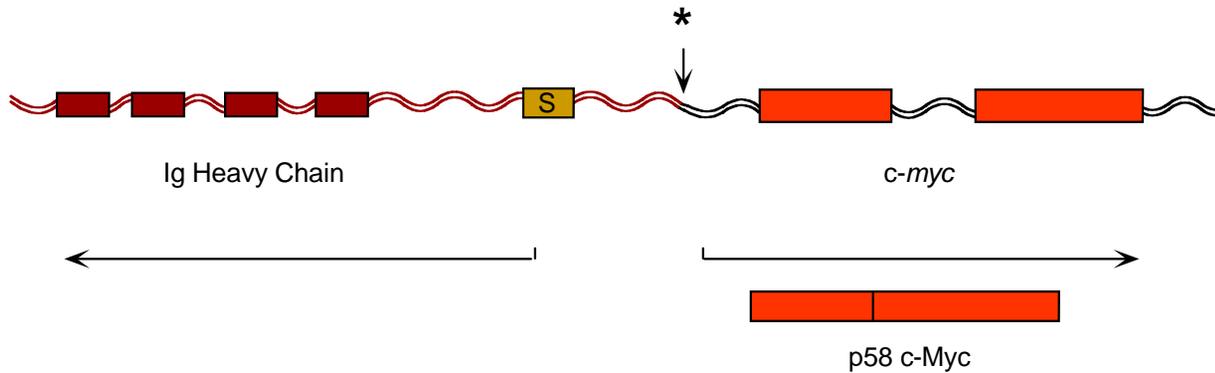


B. Proviral insertion



p58 c-Myc

C. Translocation



p58 c-Myc

Hazards Associated with Retroviruses

1. **Induction of disease in recipient.**
 - **Malignancy, immunodeficiency, etc.**
 - **Would be variable in type and of long latency, therefore difficult to detect and associate with initial infection event.**
2. **Possible additional spread of the virus to secondary recipients.**
 - **Improbable, but must be kept in mind.**
3. **Hazard is still theoretical for human vaccines, but there are examples from other bioproducts and veterinary vaccines.**
 - **HIV in clotting factors.**
 - **Reticuloendotheliosis (a gammaretrovirus) in Marek's disease vaccine.**

Again, these hazards are associated with replicating viruses, not replication defective particles.

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Sources of Retrovirus Contamination

1. Infection of donor with exogenous virus.
 - Alpharetrovirus (Avian leukosis virus) in birds
 - D-type viruses (like MPMV) in primates
 - Lentiviruses (HIV) in humans
 - Gammaretroviruses (MLV) in mice
2. Expression of infectious endogenous viruses
 - MLV, ALV, D-type
3. Expression of defective endogenous virus
 - ALV, MLV, HERV-K
4. Cocultivation with endogenous virus-producing cells.
5. Recombination between noninfectious viruses
 - Retroviral vector constructs used to make cell lines with helper or endogenous virus

Xenotropic MLV

1. Endogenous murine leukemia virus.
2. Does not infect most mouse cells due to lack of appropriate receptor, but can infect cells of most other mammals, including humans.
3. Infectious virus encoded by a single locus, *Bxv1*, found in about half of inbred strains, including ones commonly used (Balb/c, Nu/Nu, C57Bl6). Not in NIH Swiss, or cell lines like NIH 3T3.
4. A significant fraction of human tumor lines passed through nude mice are infected and produce copious quantities of virus.
5. Pathogenicity unknown, but LTR is found in recombinant, oncogenic MLV.

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Retrovirus Assays

1. Bioassay
2. Electron Microscopy
3. RT
4. PCR

Retrovirus Bioassay

- 1. The gold standard for identification of retrovirus contamination is to find virus that is infectious for an appropriate indicator cell.**
- 2. Infection can be assessed by RT or PCR assay or vector mobilization.**
- 3. Negative results are very difficult to interpret**
 - Does indicator cell have the correct receptor?**
 - Virions are quite labile and have high particle to infectivity ratios.**
 - Interference from other materials present in the vaccine.**

Electron Microscopy

1. Quite insensitive.
2. Virions can be difficult to distinguish from cell debris,
3. Can not distinguish biologically active from defective particles.
4. Many cells and cell lines are positive
 - HERV-K in human placenta and some tumors.
 - Intracisternal A particles (IAP's) in many mouse cell lines.
 - Human tumors passed through nude mice.

RT Assay

- 1. Reverse transcriptase in particles is diagnostic for retroviruses and its assay is widely used for their discovery and quantitation.**
- 2. Standard assay is only moderately sensitive and can have high backgrounds.**
- 3. PCR-enhanced (PERT) assay is very sensitive and broadly useful.**
 - Can detect and quantify the amount of RT in one virion.**
 - With some modifications, can detect all known retroviruses.**
 - Does not distinguish infectious from noninfectious particles.**

PCR Assay

1. **Can be a highly sensitive and specific way to detect and quantitate retrovirus nucleic acids.**
2. **Can also be used with broadly reactive primers to detect viruses non-specifically.**
 - **Some trade-off with sensitivity.**
 - **All cell DNA's and (probably) RNA's will test positive with broadly reactive assays.**
3. **RT PCR on pellets from cell supernatants can be diagnostic, but sequencing of product is required to identify the source of the signal.**

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Dealing with Retrovirus Contamination

1. Screening for specific agents.
2. Screening for unknown agents.
3. Removal of potential contaminating viruses.

Screening for Unknown Retroviruses

- 1. Will always succeed!**
 - All vertebrate cell substrates contain large numbers of endogenous proviruses, a few of which are occasionally expressed to yield RNA, proteins, and, often, virus-like particles.
 - For example, vaccines grown on chicken cells (or in eggs) generally have PERT and RNA positive particles derived from defective Ev-0 proviruses, of no obvious biological consequence.
- 2. Need to distinguish infectious from defective proviruses and particles.**
- 3. Polymorphism of proviruses means that we cannot assume that all individuals of a species will have the same risk of producing infectious endogenous virus.**

Removal of Retroviruses from Vaccines

1. Virions are quite sensitive to detergent and/or organic solvent treatment or other agents.
 - Some protocols are approved for certain uses (eg removal of HIV from clotting factor preparations)
2. Could be considered to add an additional measure of reassurance in some cases, but probably not where overt contamination with infectious virus has been shown.
3. Would often adversely affect the vaccine agent as well.
4. Difficult to validate.

Conclusions

- 1. Retroviruses are everywhere. All products produced by vertebrate cells will yield positive results for retrovirus nucleic acid when tested by adequately sensitive assays. In most cases, these “viruses” will represent noninfectious endogenous proviruses and their products.**
- 2. Contamination of vaccine products by any amount of known exogenous or infectious endogenous virus cannot be allowed.**
- 3. The real issue is to develop assays and approaches to distinguish the two.**