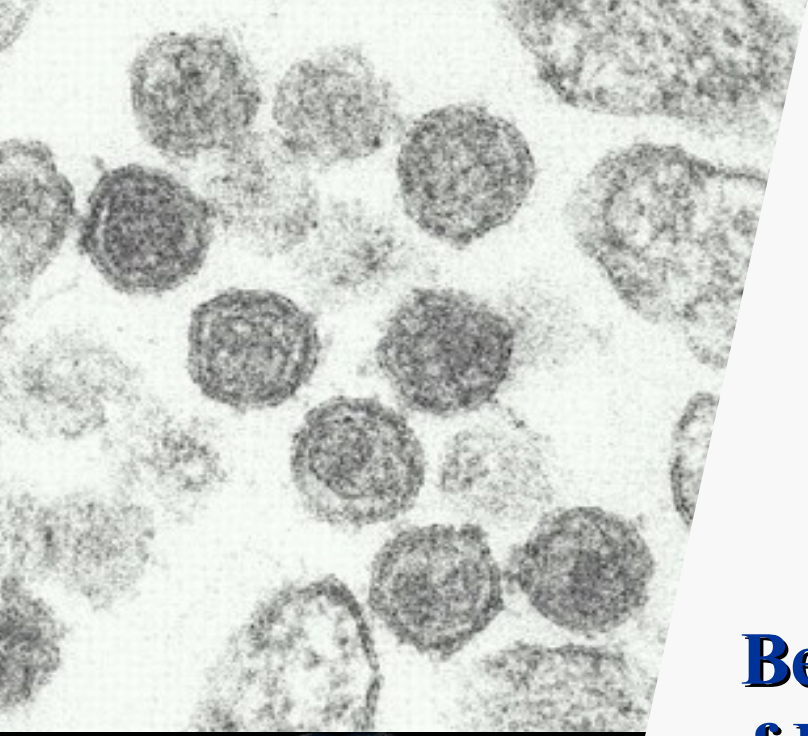

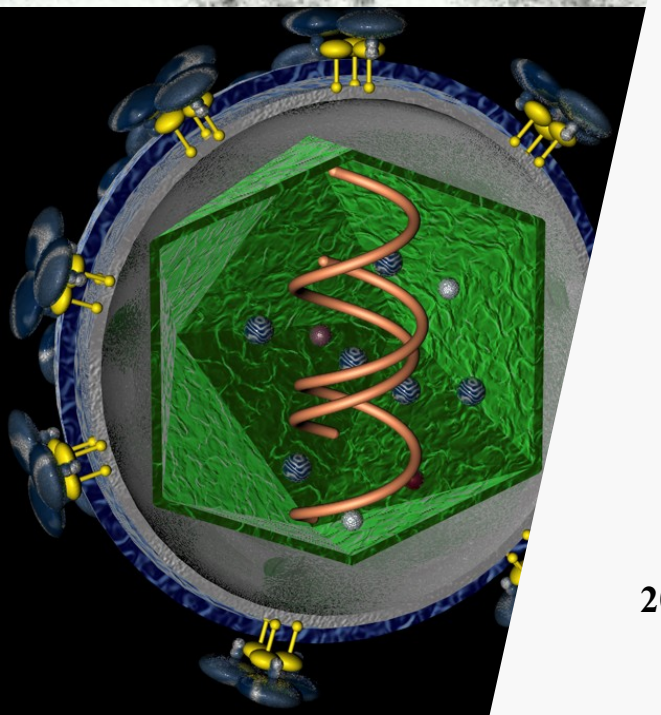

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Beneficial and Detrimental Effects of Human Endogenous Retroviruses

Reinhard Kurth,
Robert Koch Institut, Berlin
Schering Stiftung, Berlin

20th International Leukemia Workshop, Heidelberg University, July 2-3, 2011

Classification of retroelements

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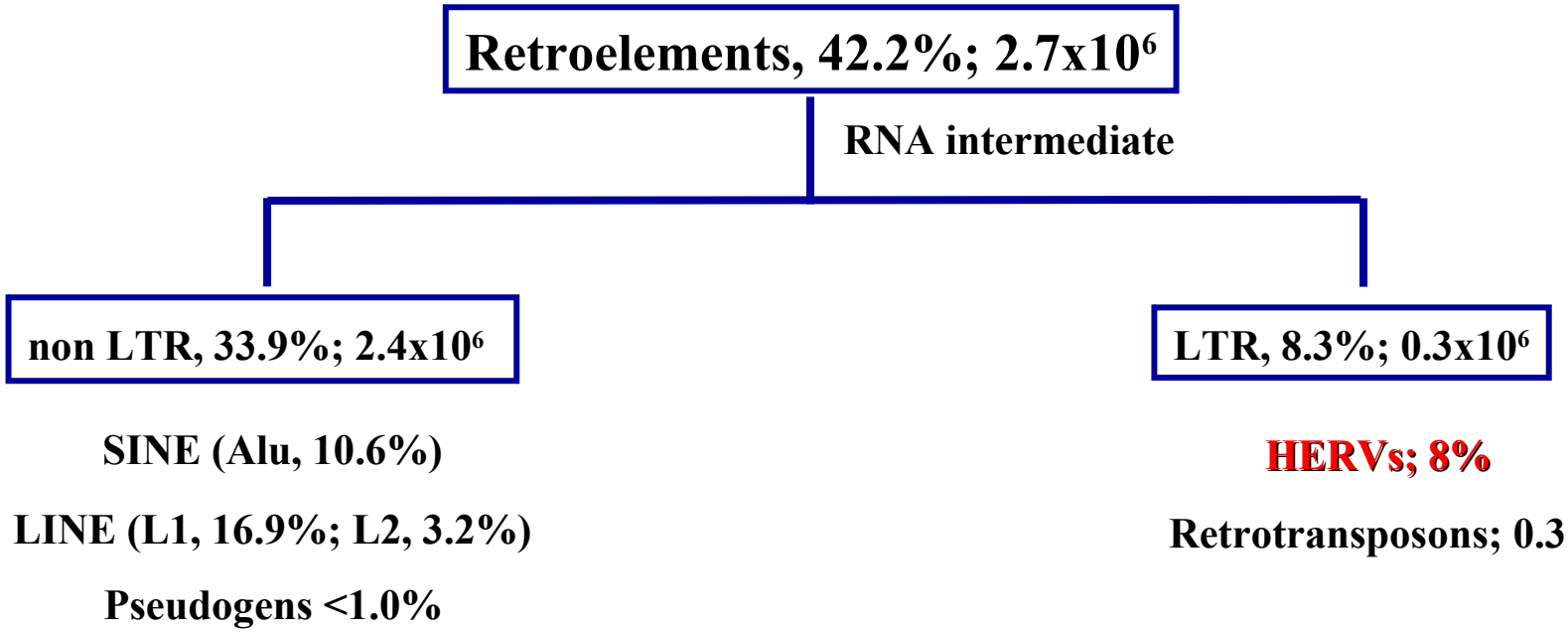

Structural Features of Retroelements

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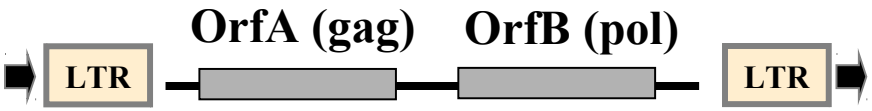
SINEs
(80-630 bp)

e.g. human Alu

LINE
(6-8 kbp)

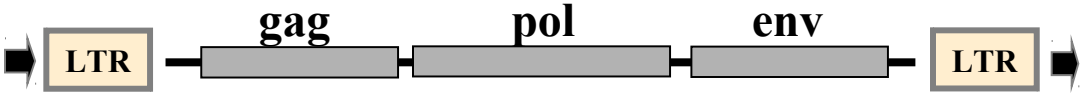
e.g. human LINE-1 (L1)

Retrotransposon
(4-8 kbp)



e.g. Drosophila Copia, Yeast Ty1

Endogenous Retrovirus
(9-10 kbp)



e.g. HERV-K

Amplification of ERVs

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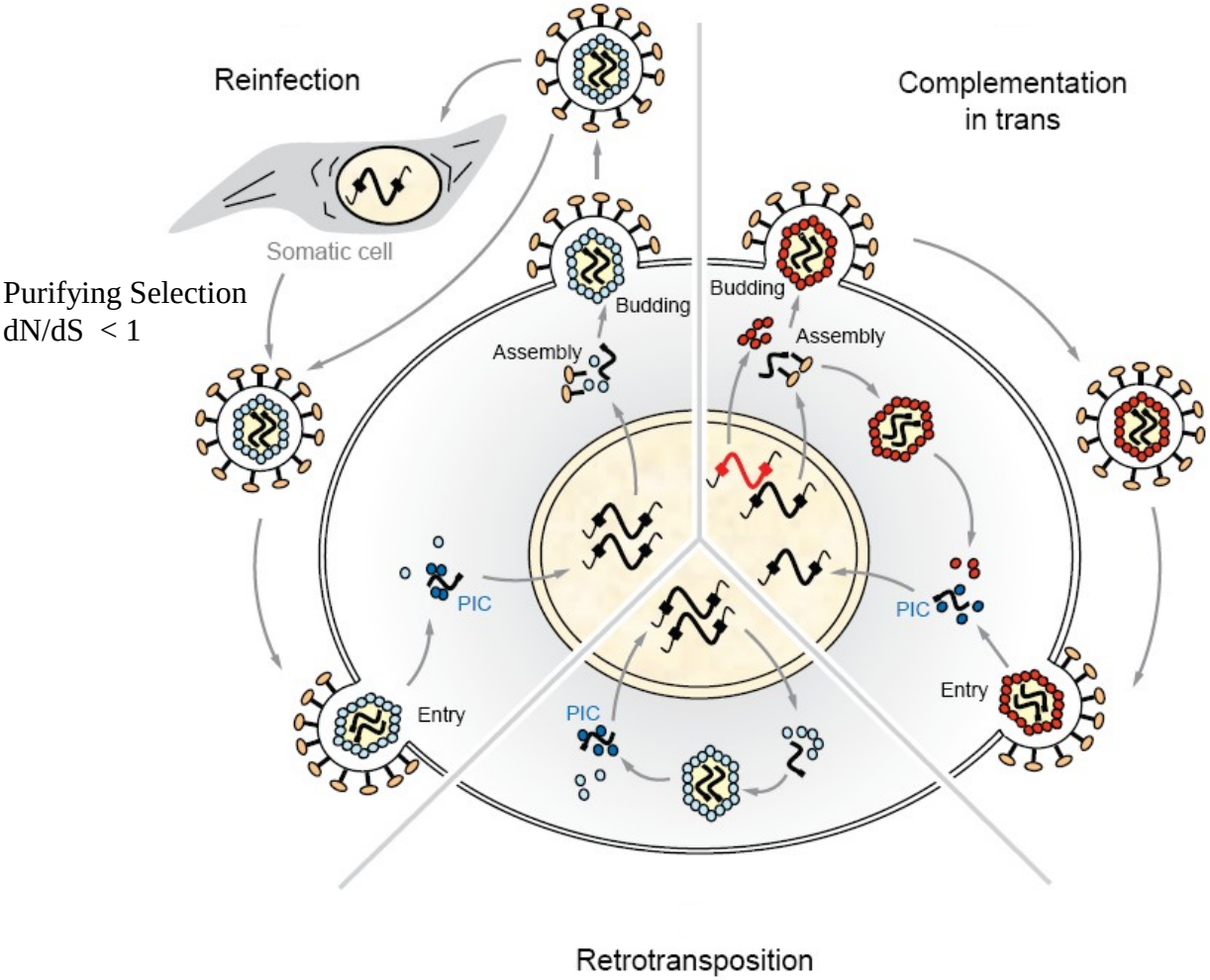

Purifying Selection
dN/dS < 1

HERV-K and primate phylogeny

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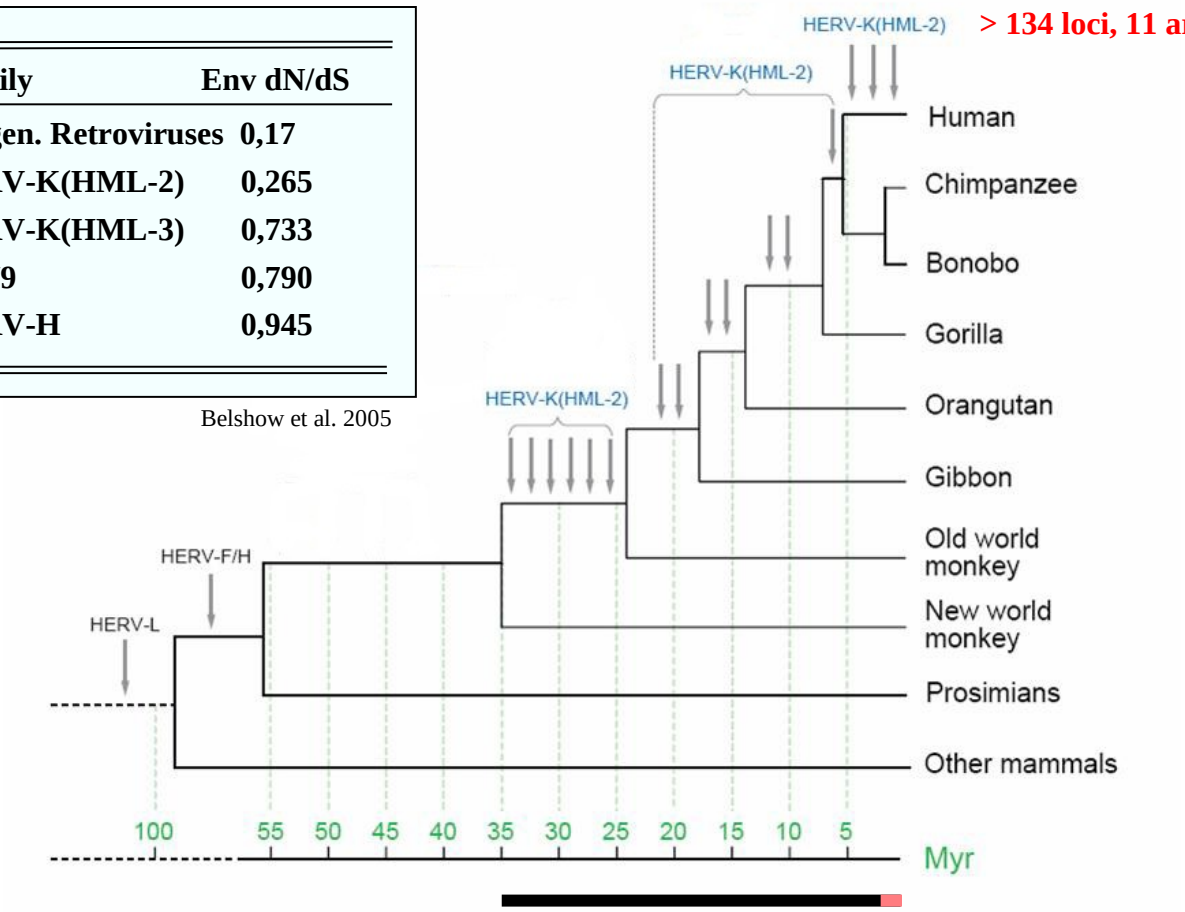
Family	Env dN/dS
Exogen. Retroviruses	0,17
HERV-K(HML-2)	0,265
HERV-K(HML-3)	0,733
ERV9	0,790
HERV-H	0,945

Belshow et al. 2005



> 134 loci, 11 are polymorphic

HERV-K (HTDV)-Particles

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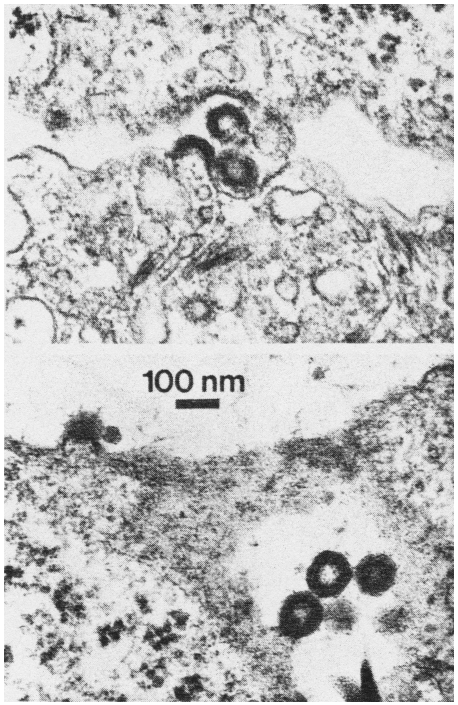
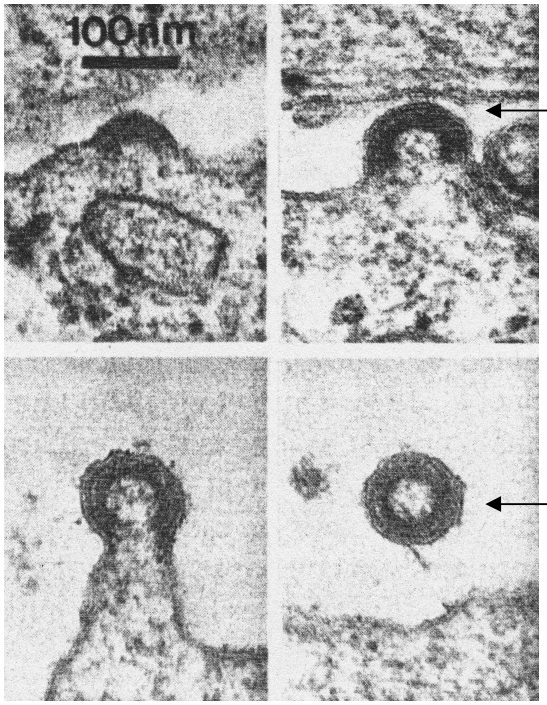


GH-Cells



budding stage

immature particle

Kurth et al. 1980

Active ERVs in Animals

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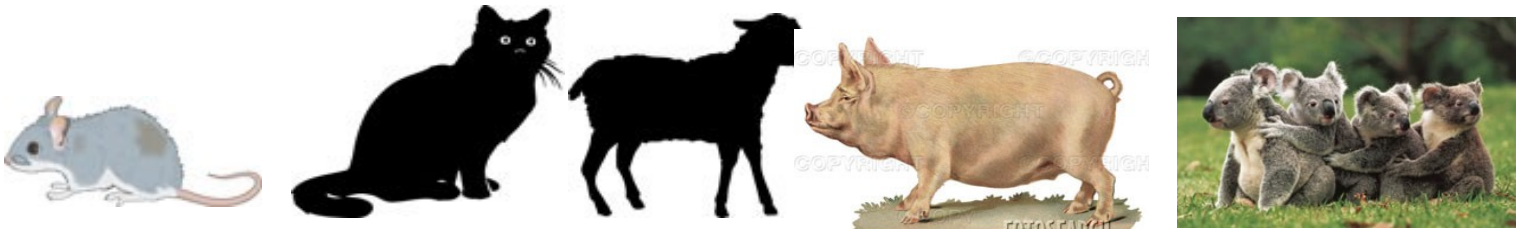
In contrast to several animals, endogenous and replicating exogenous forms of the same virus are not known in humans.
Examples:



MMTV **FeLV** **JSRV** **PERV** **KoRV**

associated with malignancies

KoRV, a new γ-Retrovirus

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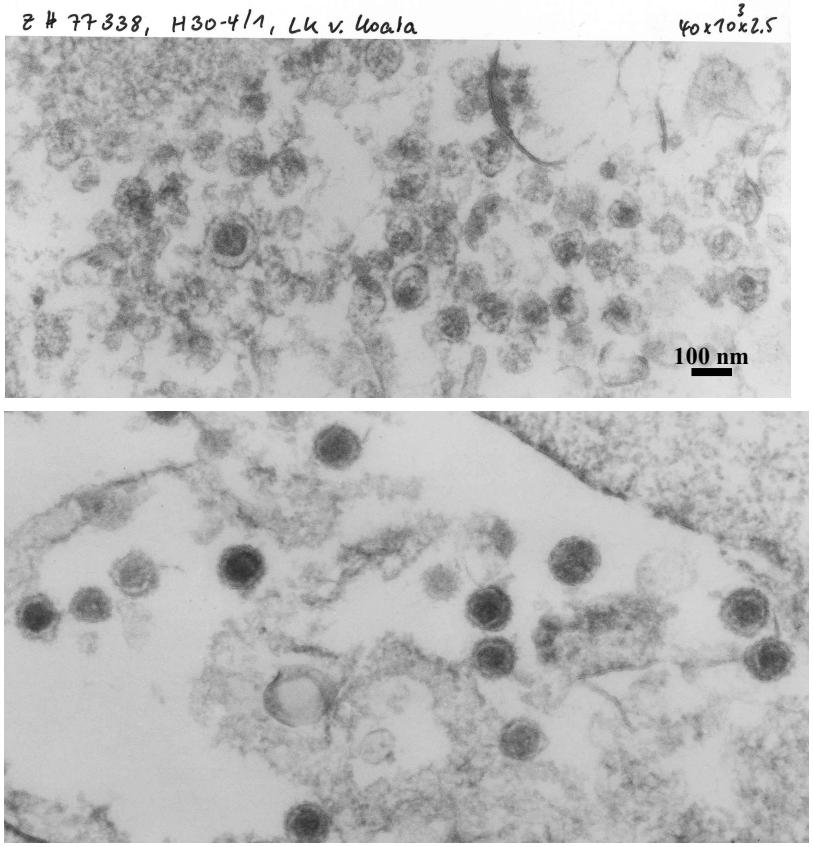
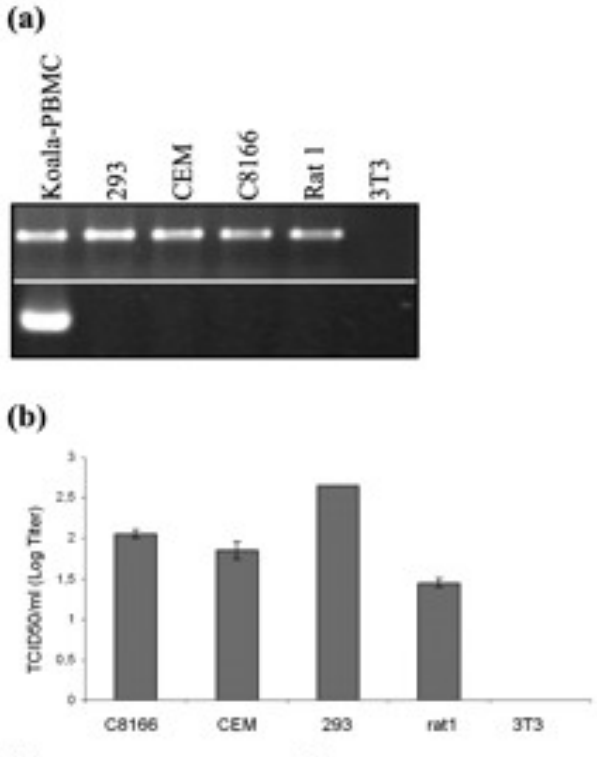
About 60% of Koalas in captivity and ca. 5 % of free living animals die of leukemia, lymphomas and virus associated immunosuppression.





KoRV replicates in human cells

Fiebig et al. *J.Virol. 2006*

Retroviral Invasion of the Koala Genome

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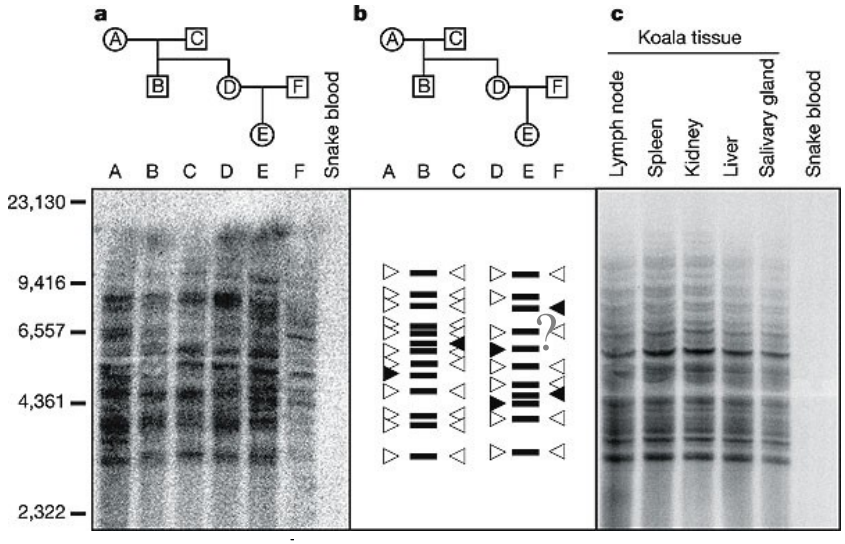
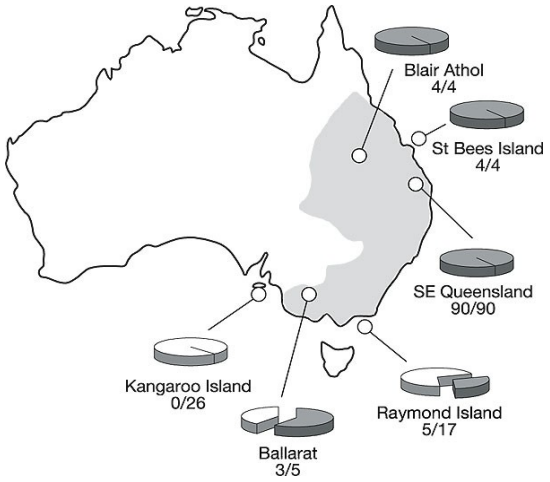


Tarlington et al., *Nature 2006*



Tarlington et al., *Nature 2006*

The Origin of KoRV

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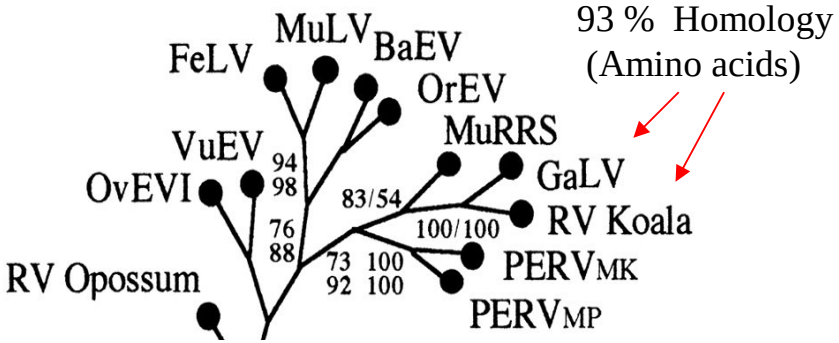

93 % Homology
(Amino acids)

< 100 years

Endogenous Retroviruses and their Biological Role (1)

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1. Consequences at the DNA level

- Chromosomal instability (rearrangements)
- Insertional mutagenesis

genome plasticity
genetic variation
regulatory elements

inactivation of genes
tissue specificity
dysregulation (malignancies)

Insertional Mutagenesis by Retrotransposable Elements

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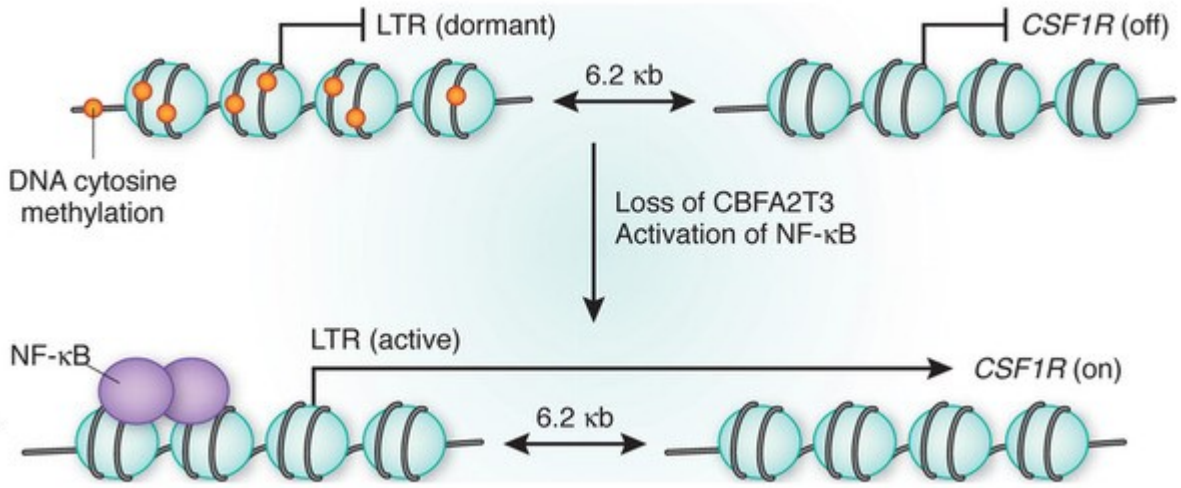
Element	Gene	Functional role	Ref.
LINE-1	Factor VIII	Haemophilia A	Landry et al. 2002
LINE-1	Dystrophin	Muscular dystrophy	Medstrand et al. 2001
SINE	Fukutin	Muscular dystrophy	Medstrand et al. 2001
Alu	NF-1	Neurofibromatosis	Wallace et al.1991
LINE-1	myc	Breast carcinoma	Morse et al. 1988
LINE-1	APC	Colon cancer	Miki et al.1992
HERV-E	Amylase	Promoteractivation	Samuelson et al.1996
HERV-K	FGFR1-K	Myeloprol. disorder	Guasch et al. 2003
HERVs	AZFa region	Male infertility	Bosch et al. 2003
Alu	GLO	Vitamin C	Challem et al. 1998

Derepression of LTRs and activation of proto-oncogenes

ROBERT KOCH INSTITUT

Demethylation and activation of an LTR upstream of the Colony Stimulating Factor 1 Receptor in Hodgkin´s lymphoma

Lamprecht et al., Nat. Med. 2010



Endogenous Retroviruses and their Biological Role (2)

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2. Consequences at the protein level

Reverse transcriptase
- Formation of pseudogenes, retrotransposition

Malignant transformation
- Rec, Np9, others ?

Autoimmunity, superantigenes
- Gag, Env

Core protein Gag,
- Protection by Fv-1-related factors

Surface envelope glycoprotein (SU-Env)
- Protection by receptor interference

Transmembrane envelope protein (TM-Env)
- Fusion peptide: syncytiotrophoblast

Immunosuppression and tumor promotion *in vivo* by retroviral transmembrane envelope proteins

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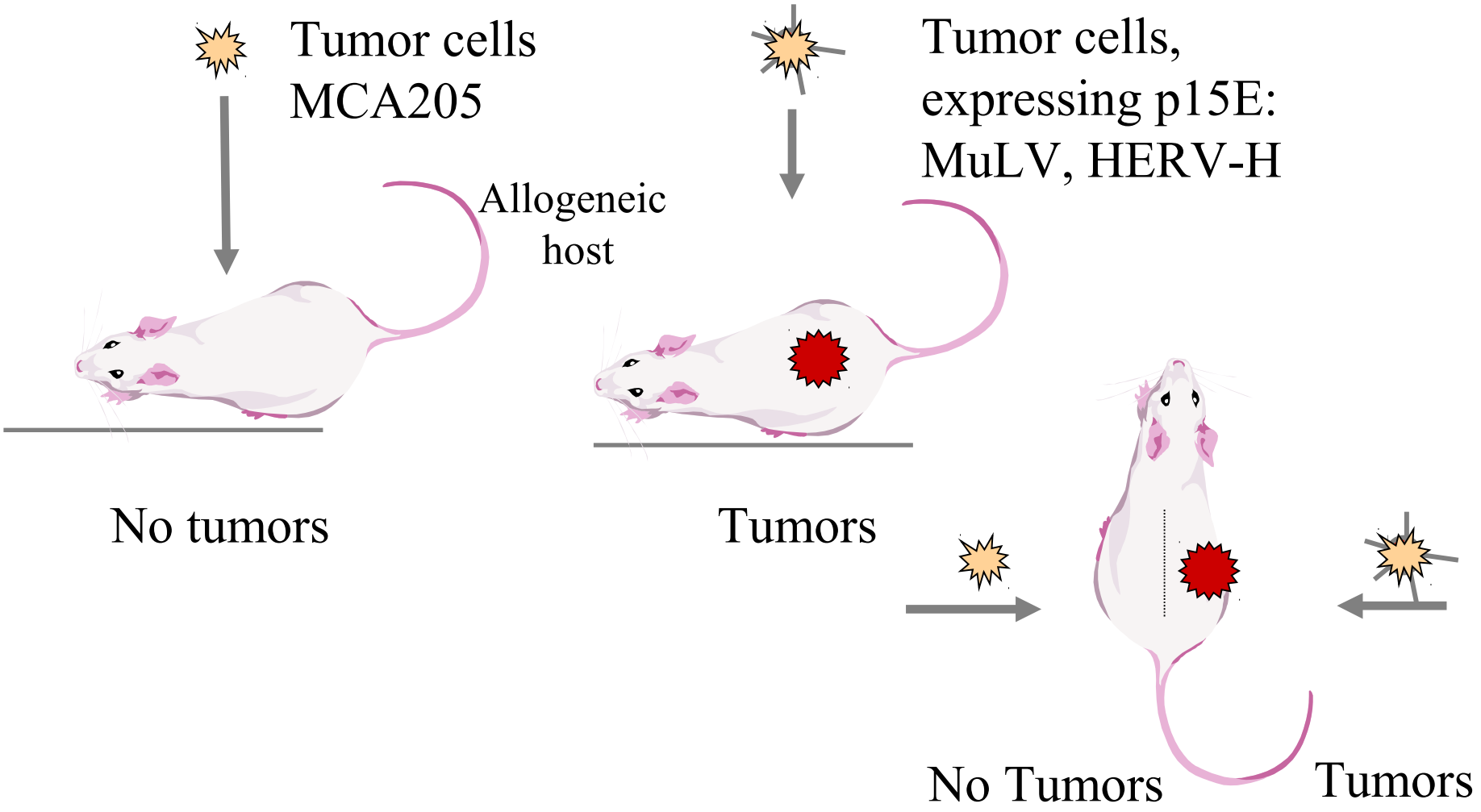
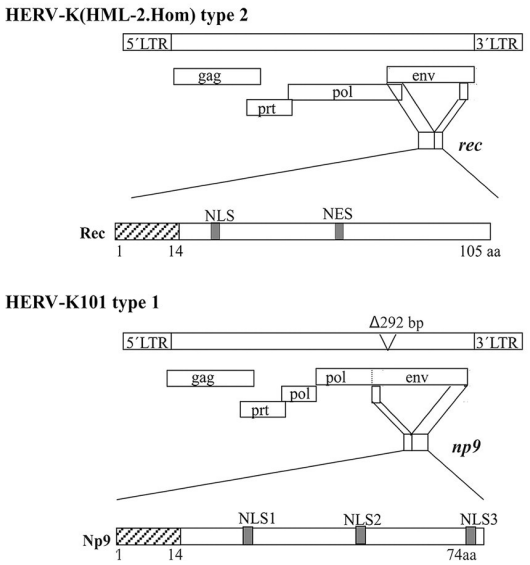


Mangeney & Heidmann, 1998; Blaise et al., Mangeney et al., 2001

Rec (cOrf) and Np9



Denne. et al., 2007

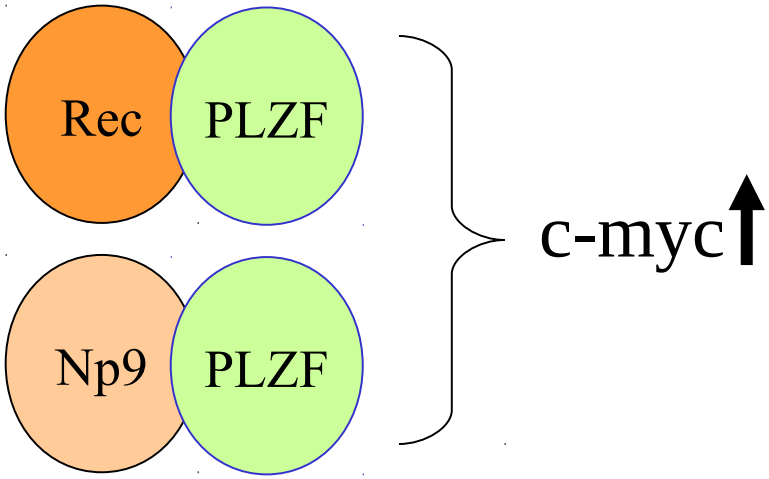
- Expressed in teratocarcinomas and other germ cell tumors, Np 9 in breast cancer cells
- Localization in the nucleolus, Nuclear Localization Signal (NLS)
- Rec: shuttle of unspliced viral mRNAs out of the nucleus
- Np9: mutant due to a deletion and abnormal splicing

Oncogenic evidence for Rec:

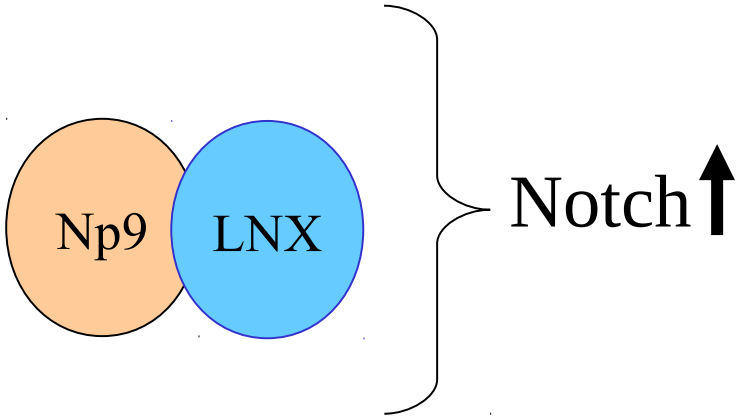
- Tumors in nude mice after injection of transfected mouse cells

- “Carcinoma in situ” in transgenic mice expressing Rec

How Could Rec and Np9 Induce/Promote Cancers ?



Denne, *J. Virol* 2007



Armbruester, *J. Virol* 2004

PLZF (promyelocytic zink finger protein):

- tumor suppressor
- repressor of ***c-myc*** transcription
- implicated in some forms of APL
- critical for spermatogenesis (mice)
- abnormal spermatogenesis is thought to predispose humans to the development of germ cell tumors

LNX (Ligand of Numb X):

- regulates expression of Notch

Notch:

- causative for mammary-tumors following MMTV integration
- associated with breast and germ cell tumors and leukemia in humans
- part of the Ras signaling pathway

Rec is expressed in malignant cells and interacts with the short glutamine-rich tetratricopeptide (hSGT)

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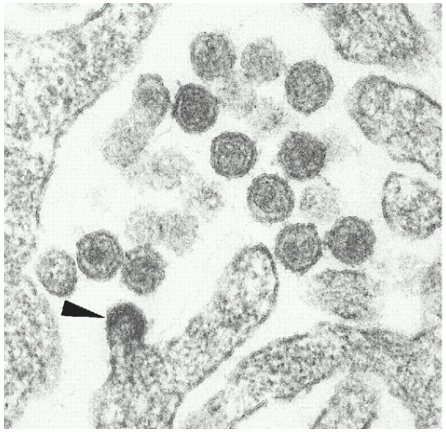
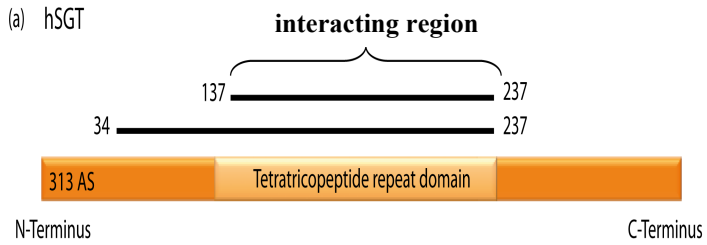
HERV-K expression in tumor cells (Teratokarzinoma)



Bieda et al. 2001



hSGT binds to the androgen receptor (AR) and negatively regulates its activity

Rec activates androgen dependent promotors

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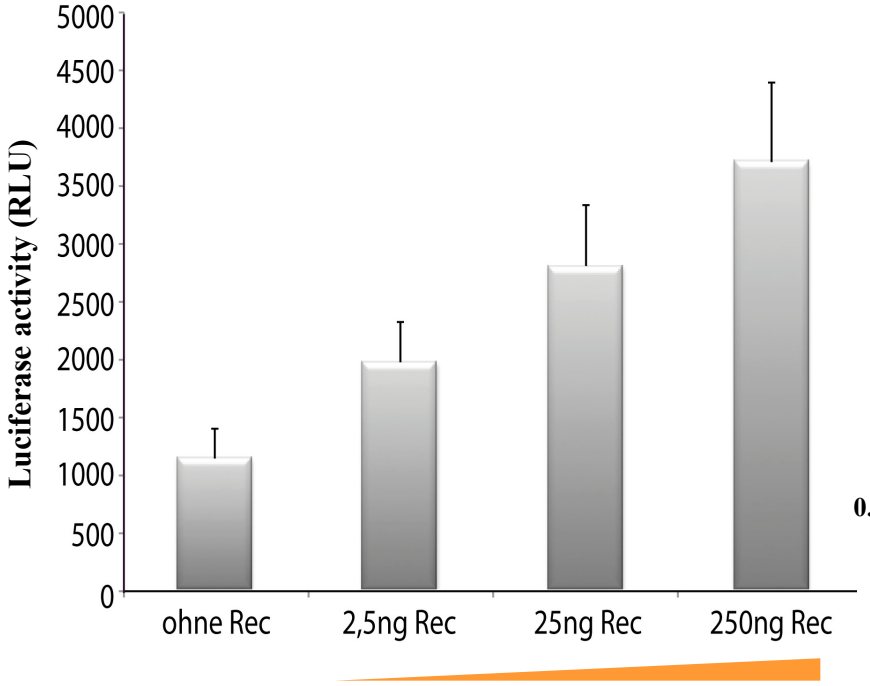

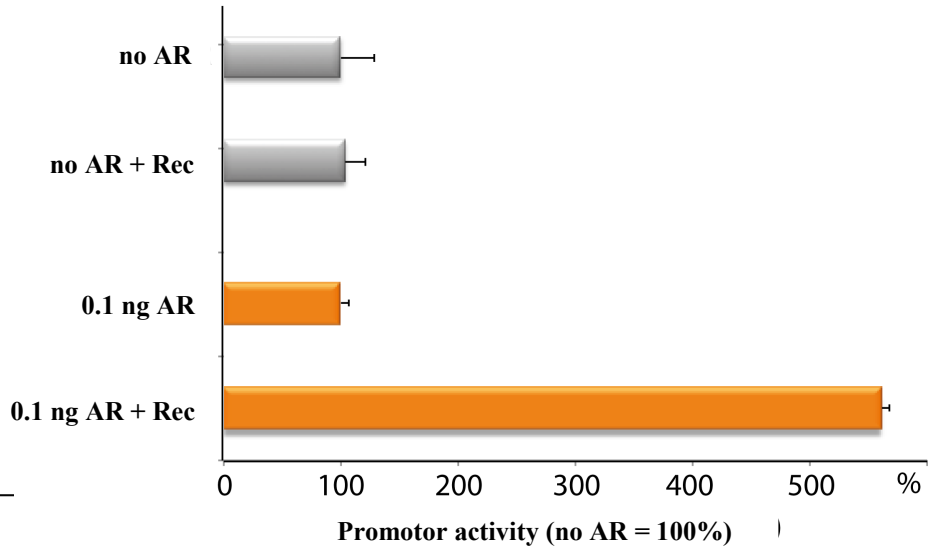

LNCaP (Prostate, AR+)

DU145 (Prostate, AR-)

Model: Rec-associated tumor promotion

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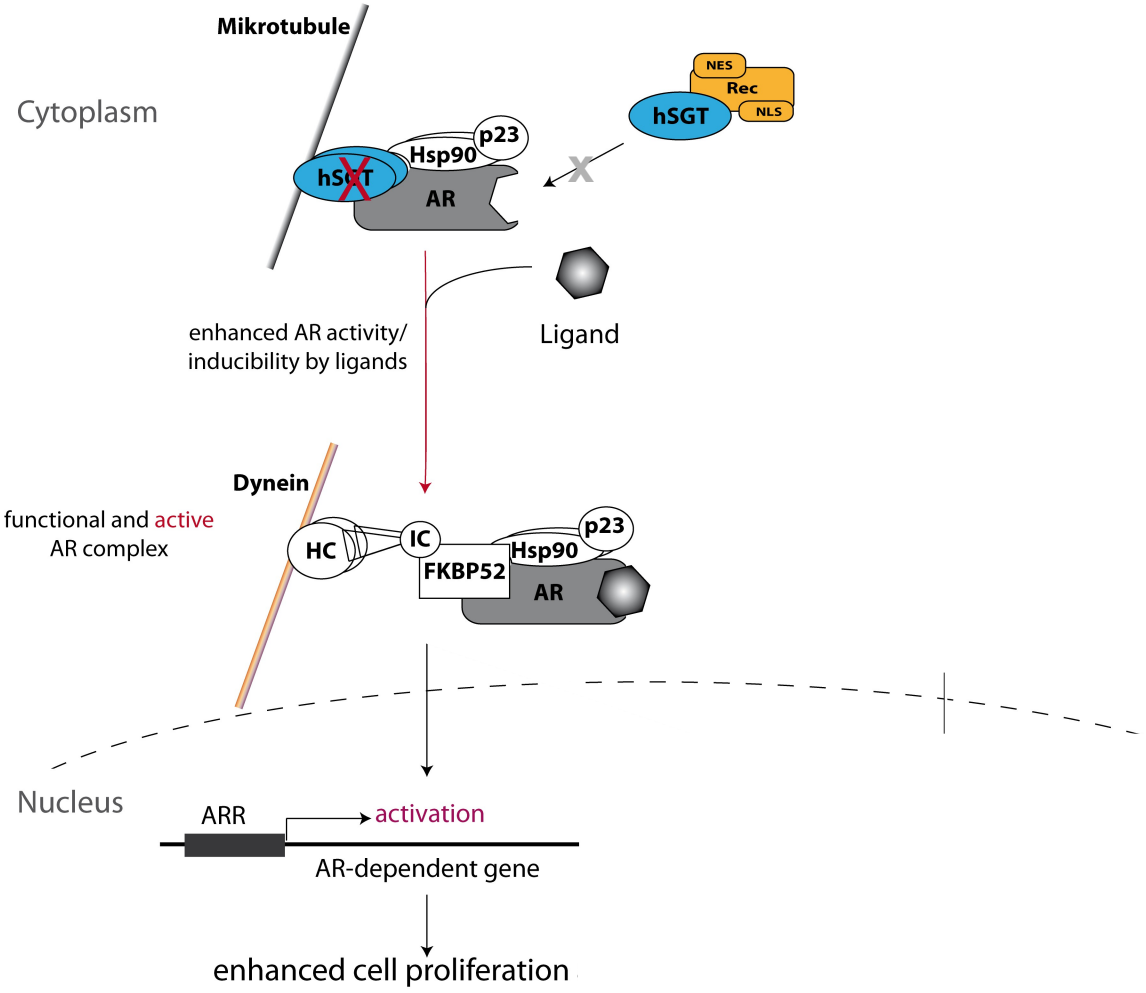

The Env-Protein of HERV-W

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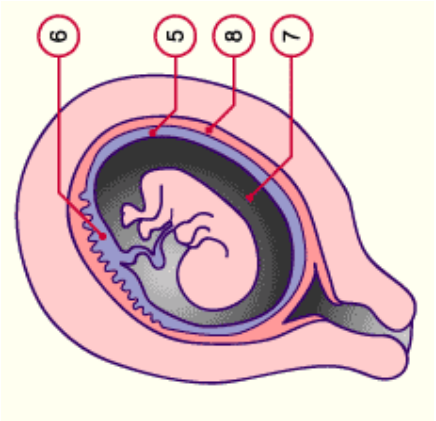
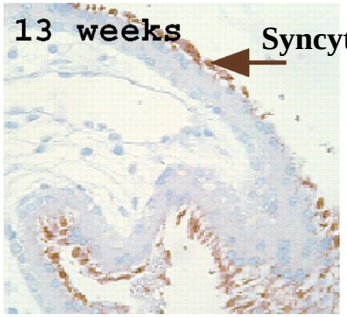
- Syncytin is an HERV-W transmembrane envelope protein
- Major site of expression: placental syncytiotrophoblast
- Expression of recombinant syncytin: Formation of giant syncytia
- Downregulation of syncytin expression and abnormal protein localization has been observed in pre-eclampsia (sub-optimal invasion of trophoblast)
- Has an immunosuppressive domain

Endogenous JSRV Env is required for the generation of the Trophectoderm in sheep



6 - Chorion
7 - Amnion
8 - Dezidua



Frendo et al. 2003

Do we harbor a replication competent HERV in our genomes ?

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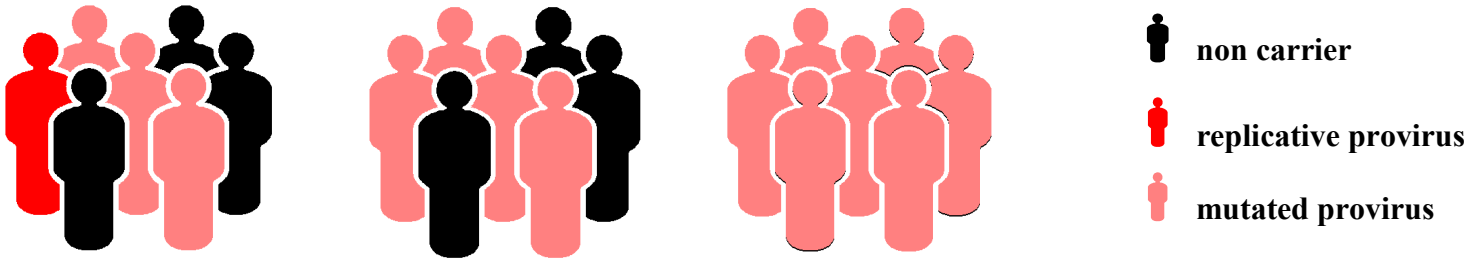
- No completely preserved HERV was found in the human genome (Lander et al. 2001)
- Are replication competent recently acquired HERV-K proviruses present in a few humans and how functional are they?



HERV-K113

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- discovered in a human genomic BAC-library (Turner et al. 2001)
- integrated on chromosome 19p13.11
- intact open reading frames for all proteins
- almost identical LTR-sequences
- conserved functional motifs
- allelic polymorphism

HERV-K113: Prevalence

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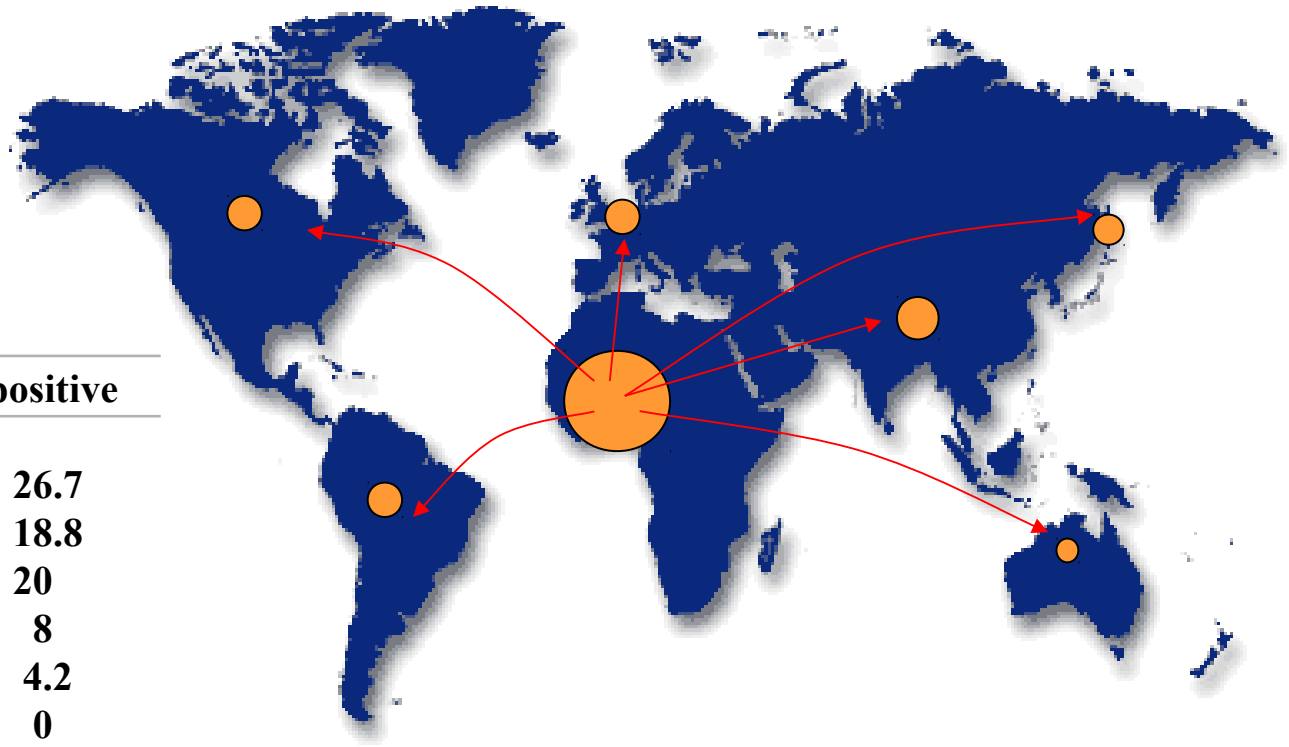

Country	n	positive
Malawi	60	26.7
Cote d´Ivorie	64	18.8
Kenya	50	20
Yemen	50	8
UK	96	4.2
Papua N. G.	54	0

Moyes et al. 2005

⟹ African origin

Generation of a HERV-K113 molecular clone

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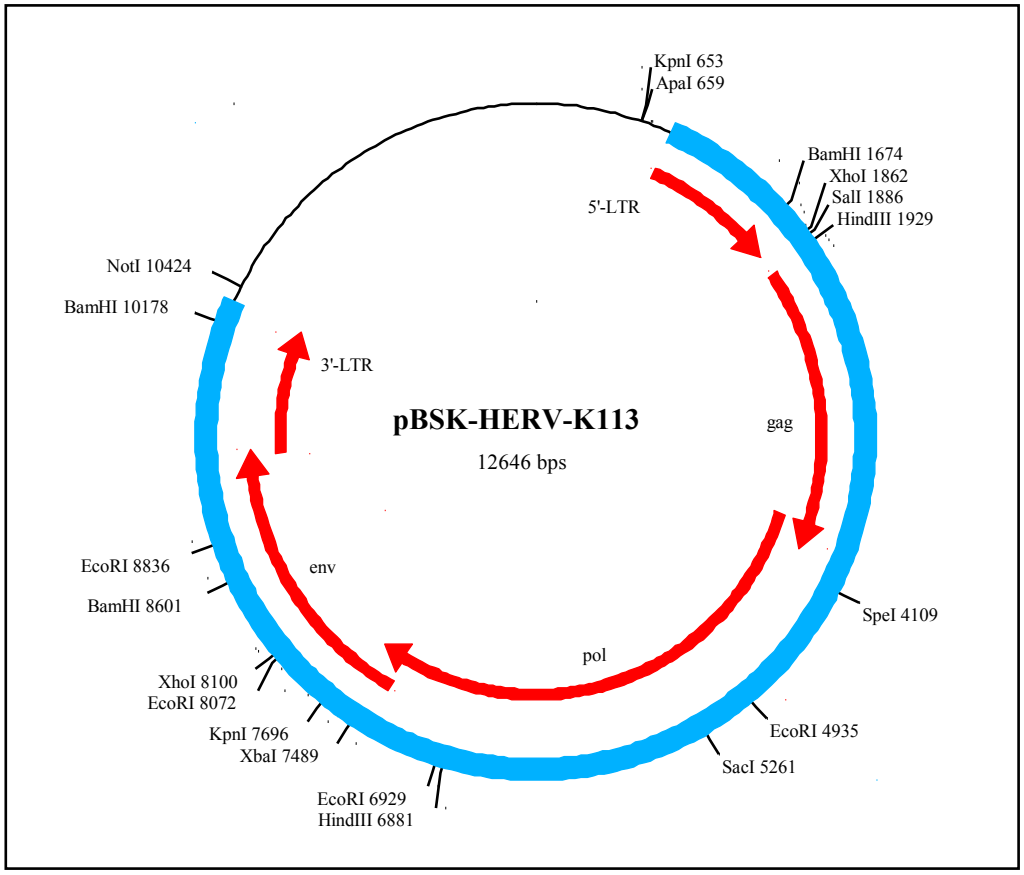

Reversion of postinsertional mutations

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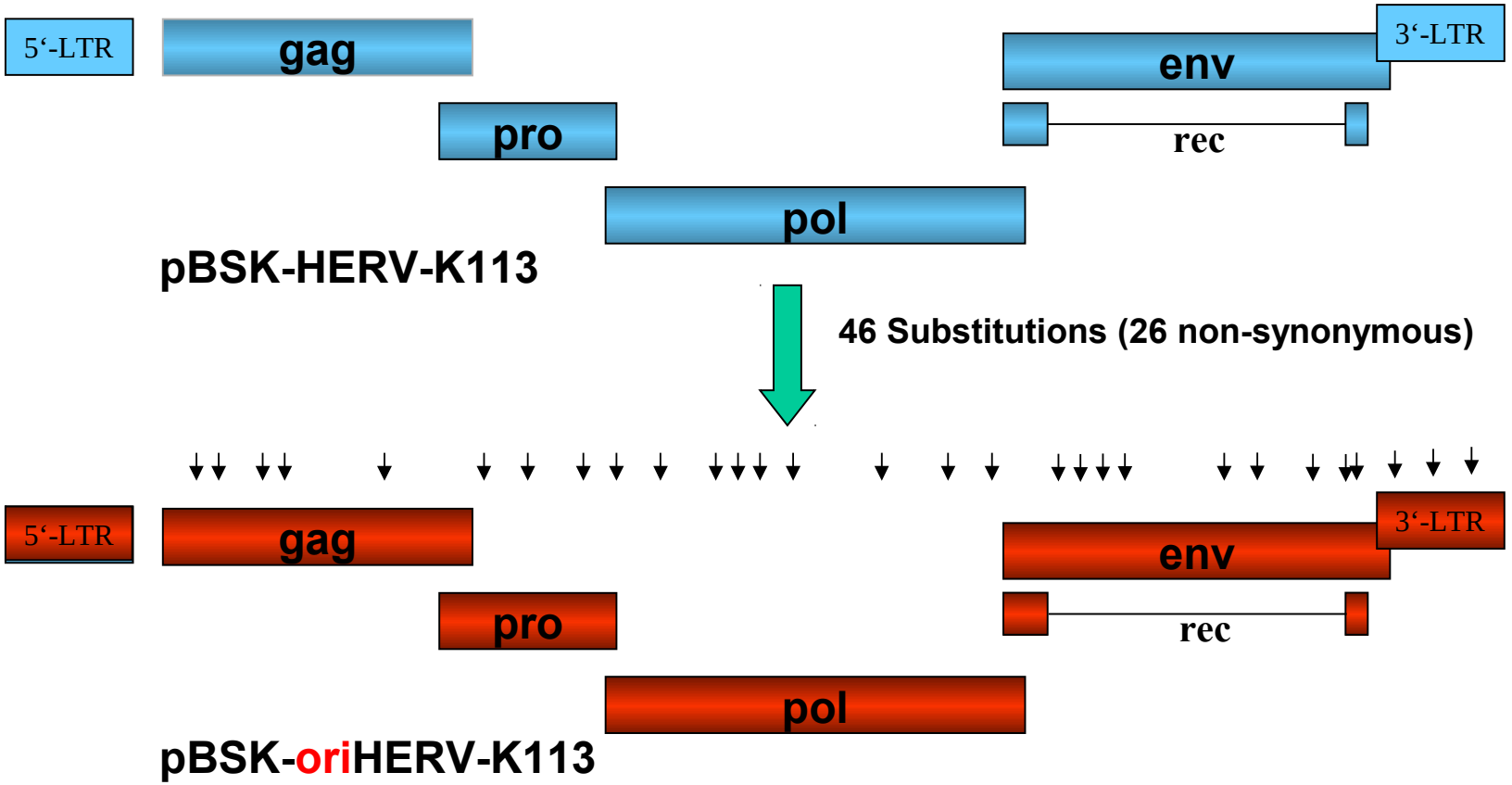
K101, K102, K104, K107, K108, K109, K115, Y178333, AP000776, AC025420



Morphology of oriHERV-K113 virions

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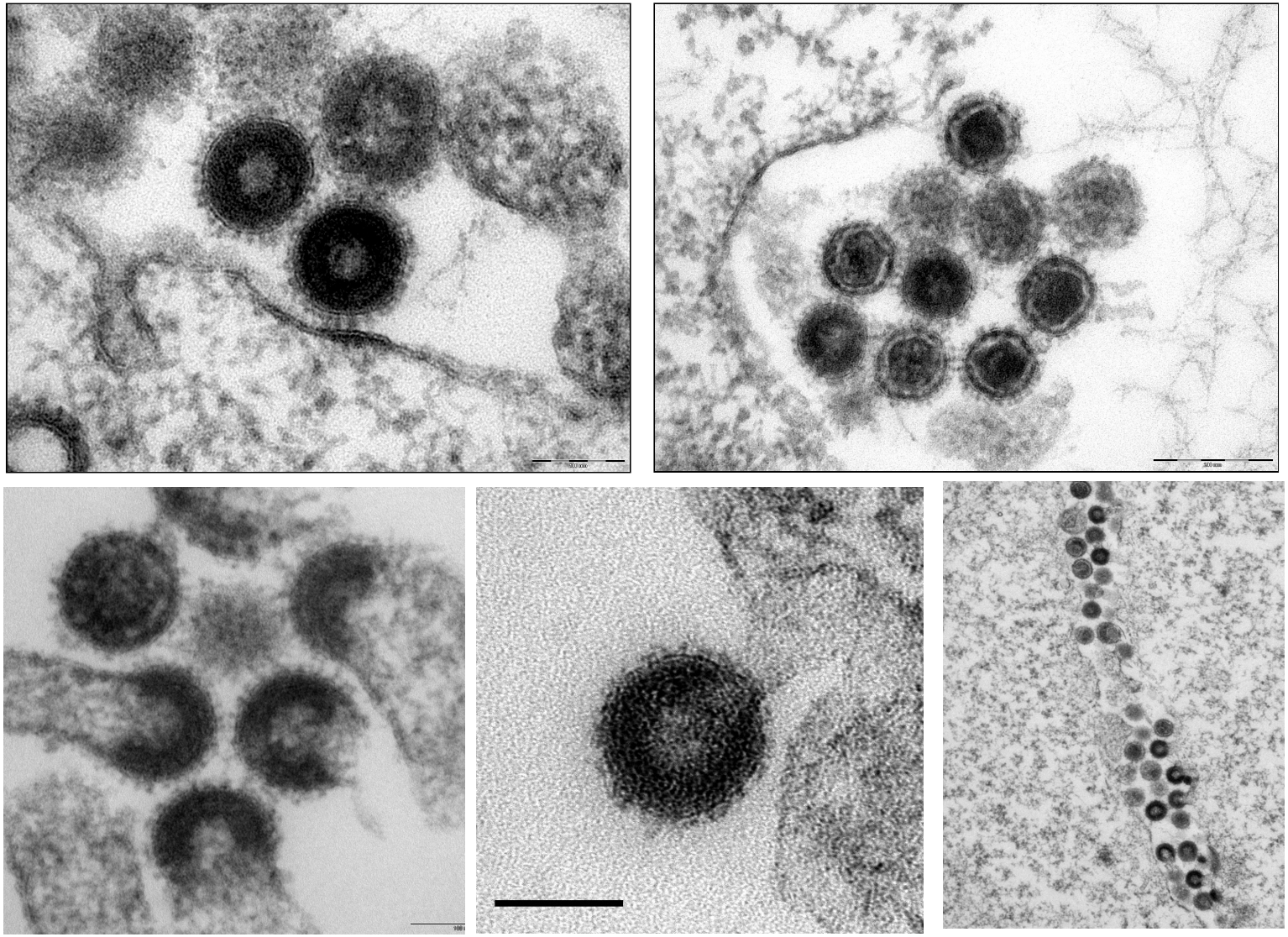

The Envelope Protein of HERV-K can pseudotype heterologous retroviruses

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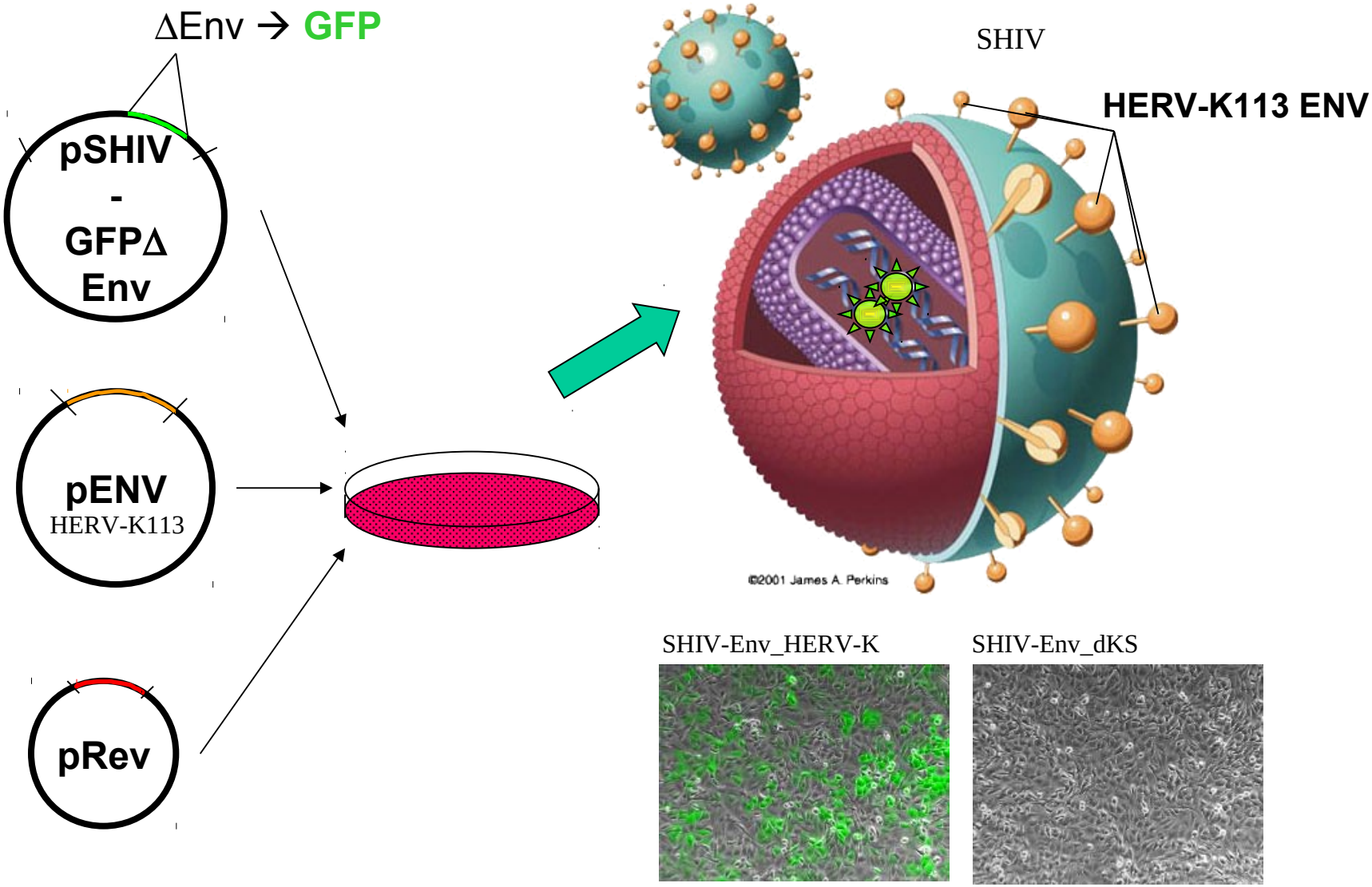
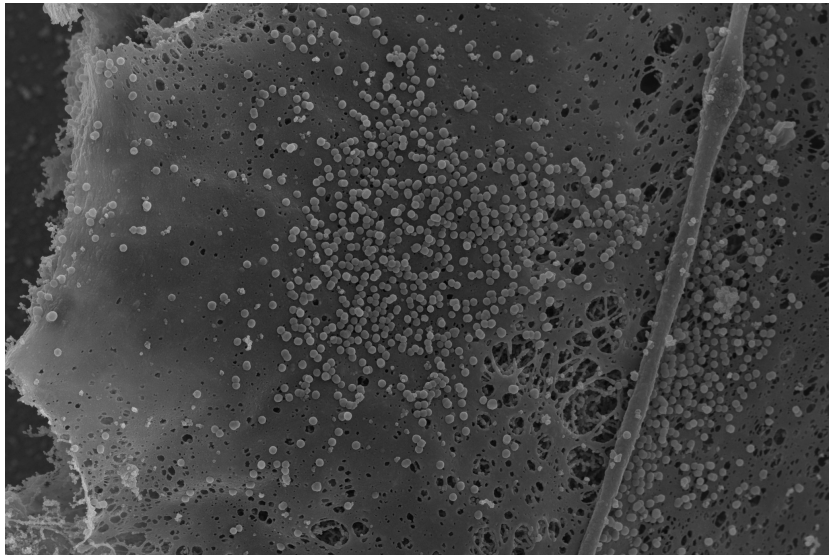


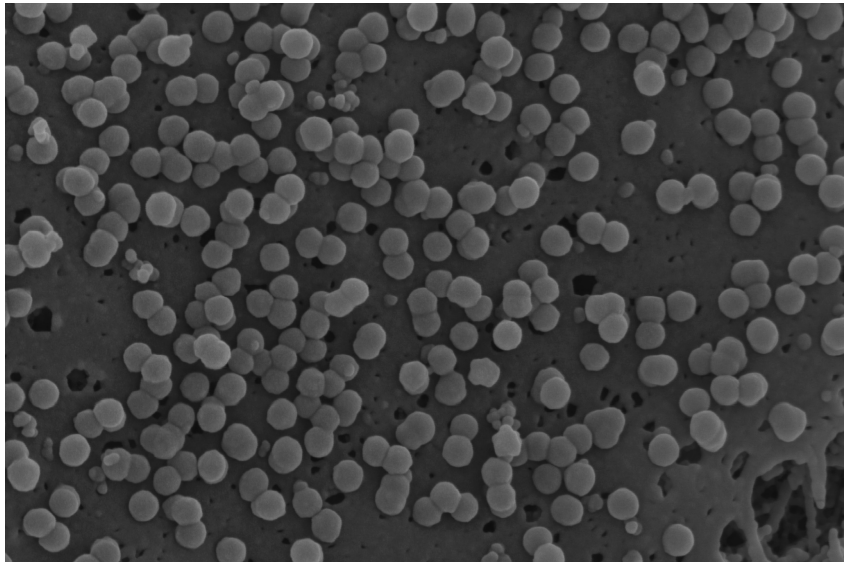
Summary

- Retroelements constitute 42% of our genomes. About 8% are retroviral sequences of ancient origin.
- HERV integration has increased the plasticity of our genomes and does influence the expression and regulation of cellular genes.
- Insertional mutation and HERV proteins (Rec, Np9 and Env) may cause cancer and autoimmune diseases
- Some HERV proteins are under purifying selection because they seem to benefit the host (e.g. Syncytin in placenta formation)
- Reconstituted HERVs are very useful to study particle morphology, their pathogenic potential as well as cellular factors restricting retroviral amplification.

Acknowledgments

Norbert Bannert, Joachim Denner,
Nadine Beimforde, Kirsten Hanke,
Robert Koch-Institut, Berlin





HEL 293T cell expressing HERV-K Particles