

EIAV as a Research Model for other Lentiviruses: Lessons learned and to be learnt.

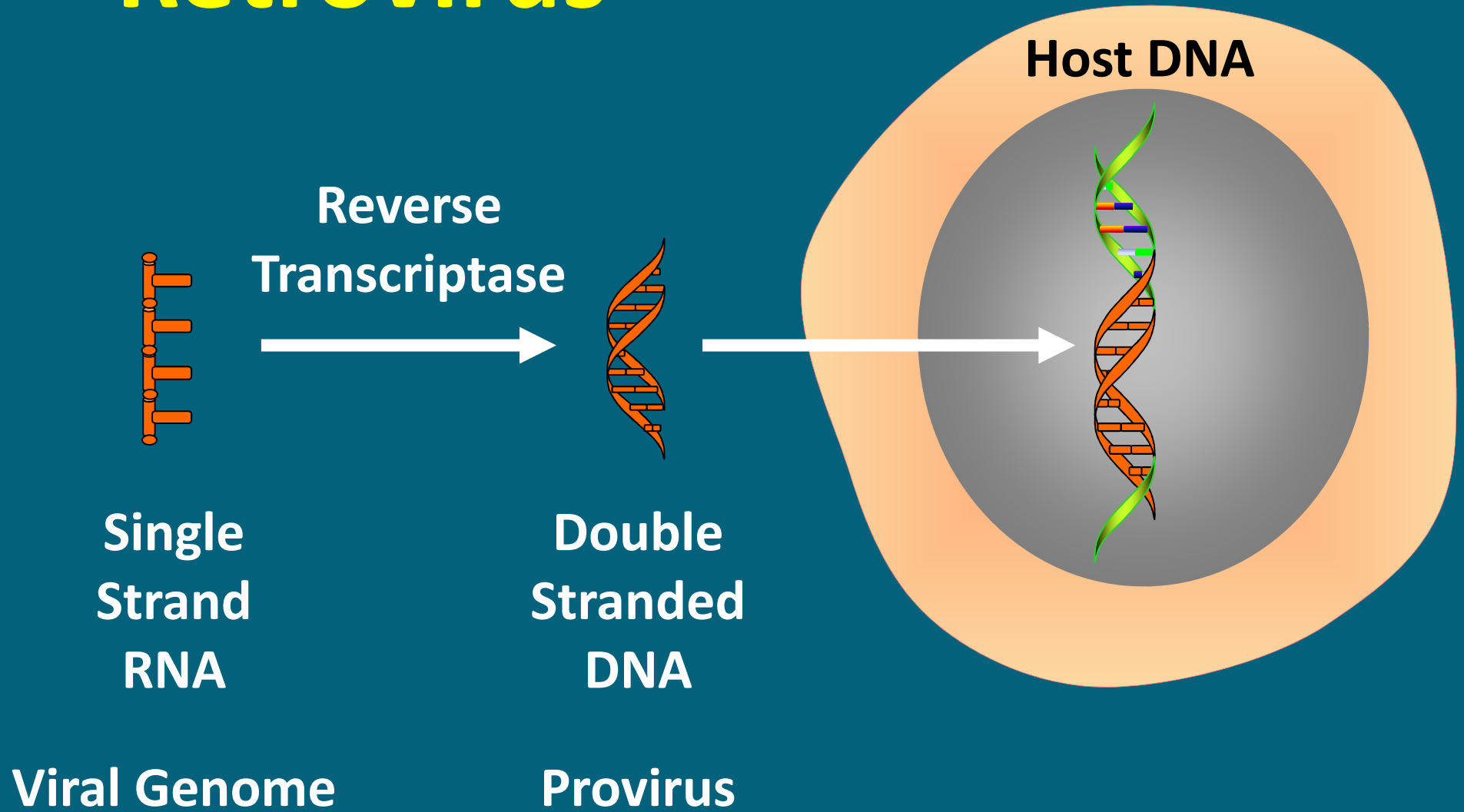


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What is a Lentivirus?

- Family: Retroviridae
- Subfamily: Orthoretrovirinae
- Genus: Lentivirus

Retrovirus

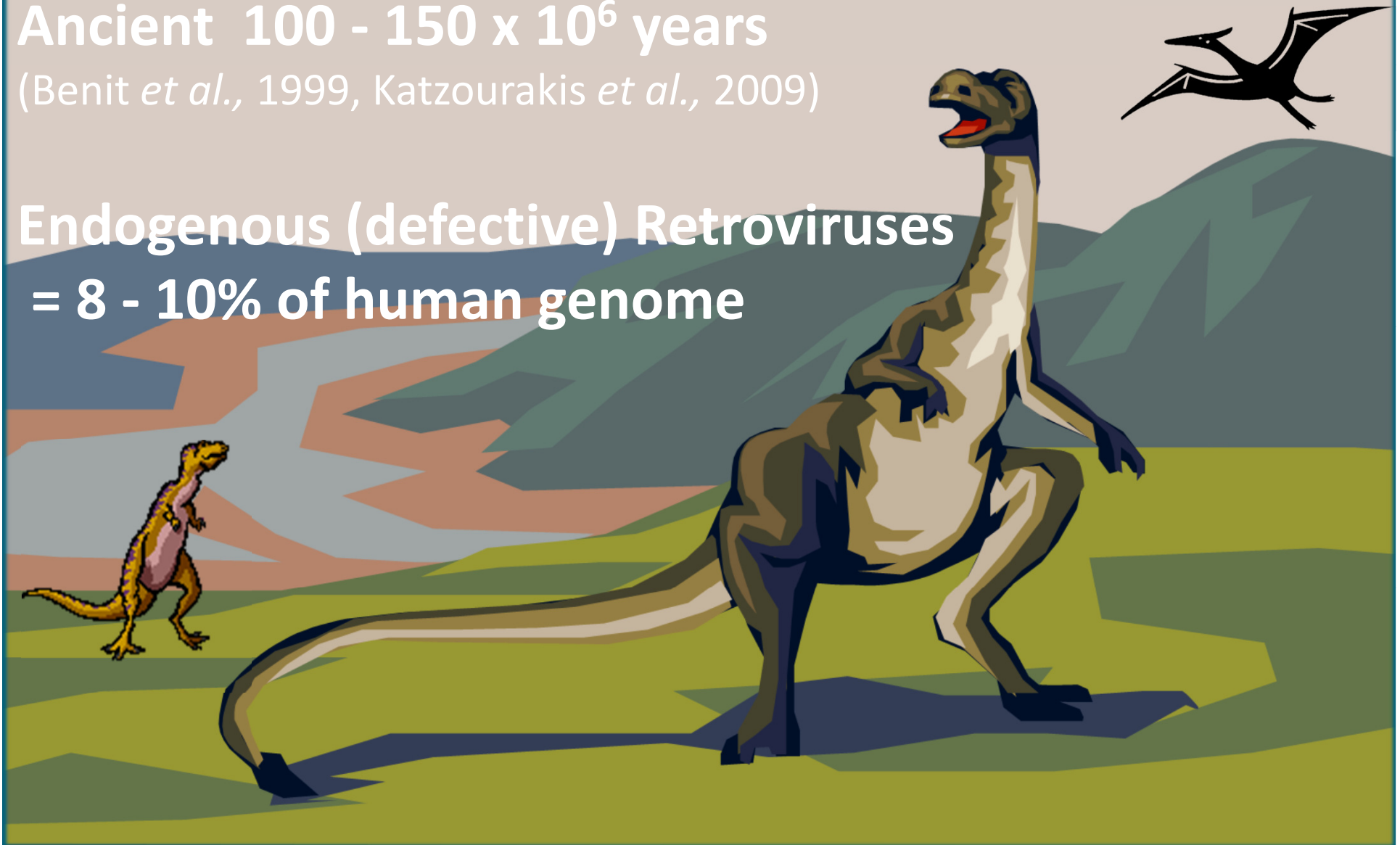


Retroviruses

Ancient $100 - 150 \times 10^6$ years

(Benit *et al.*, 1999, Katzourakis *et al.*, 2009)

Endogenous (defective) Retroviruses
= 8 - 10% of human genome



Retroviruses

LTR

GAG

POL

ENV

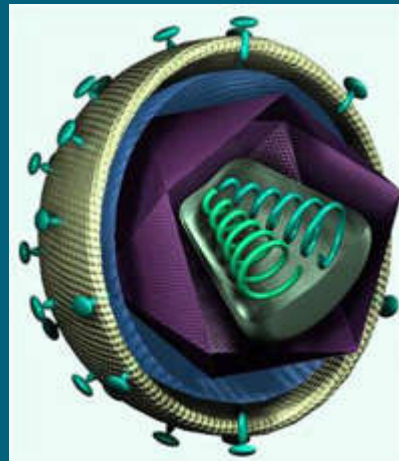
LTR



Structural Core
Proteins
p15, p26, p11, p9

Replicative
Enzymes

Envelope Surface
Glycoproteins



Host Defenses

Immune Response:

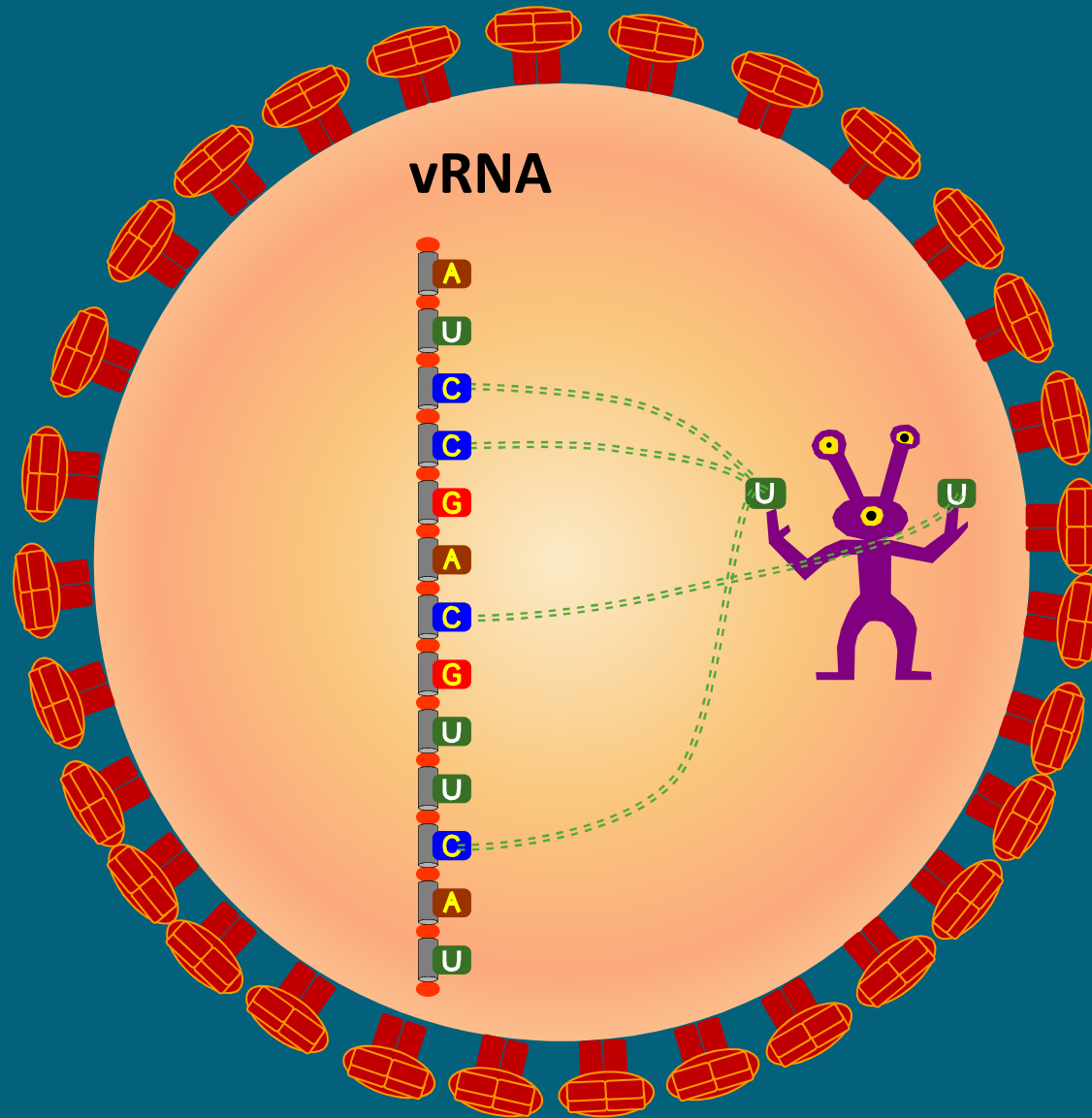
Innate

Adaptive

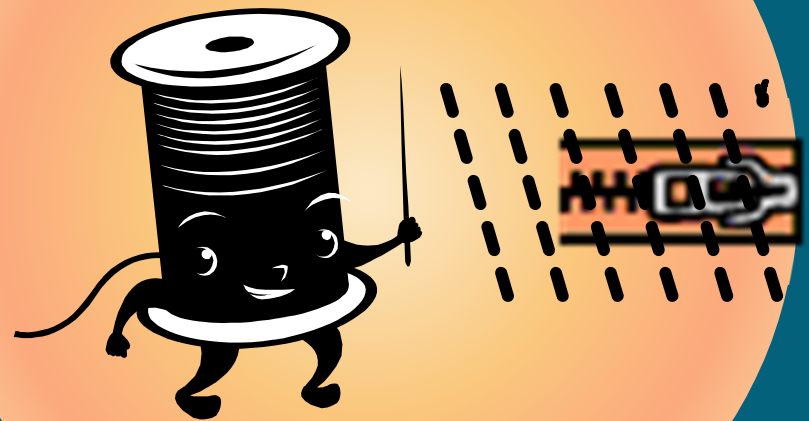
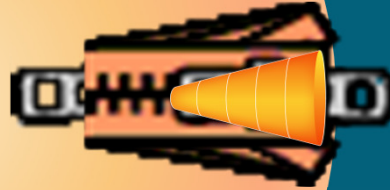
Retroviral Restriction Factors:

- **Apolipoprotein β Editing Complex 3 (APO β EC3)**
- **Tripartite Motif-Containing Protein 5 α (TRIM5 α)**
- **Tetherin**

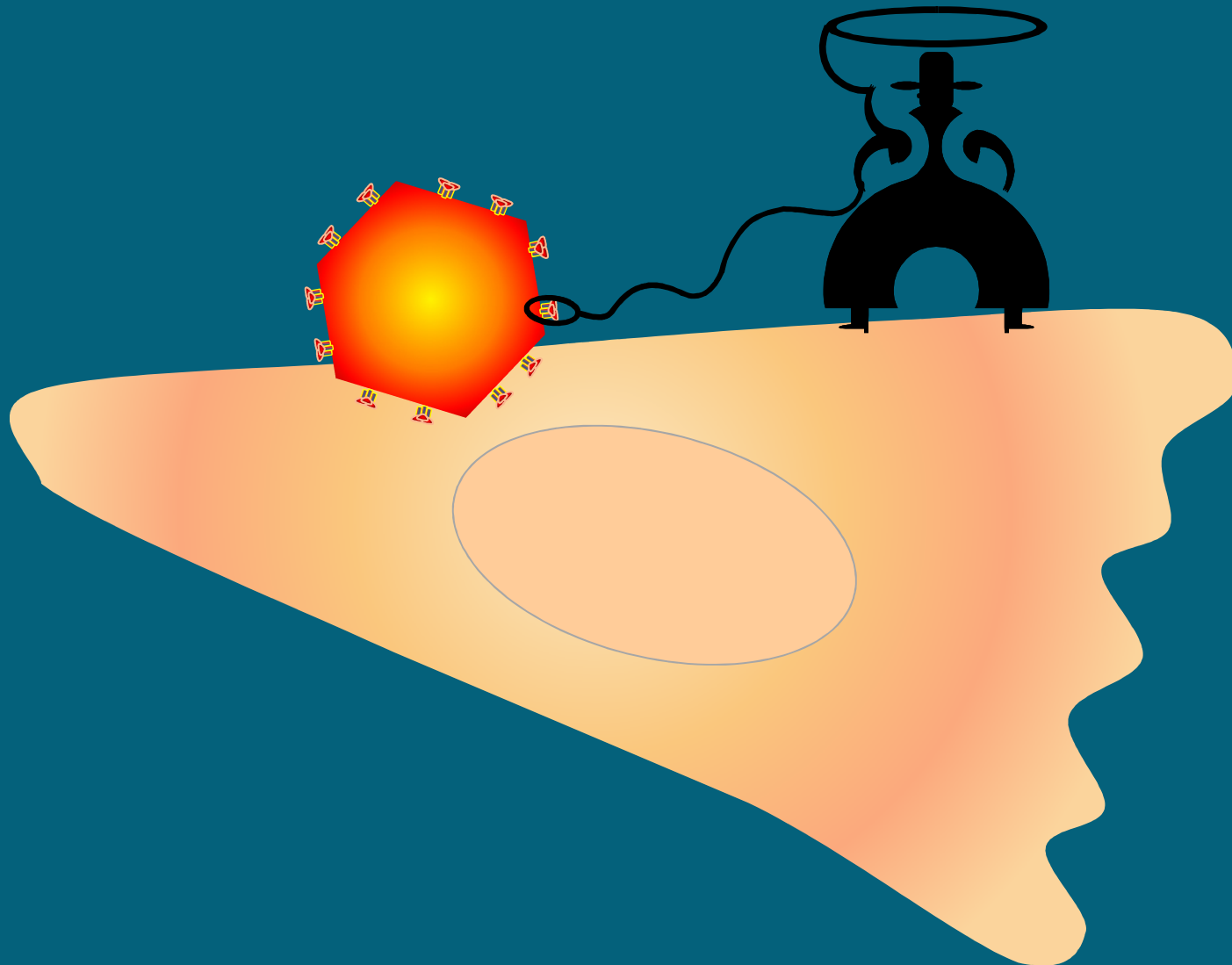
APOBEC3 (Cytosine Deaminase)



TRIM 5α



Tetherin



Lentiviral Characteristics

Genetically, Morphologically Distinct

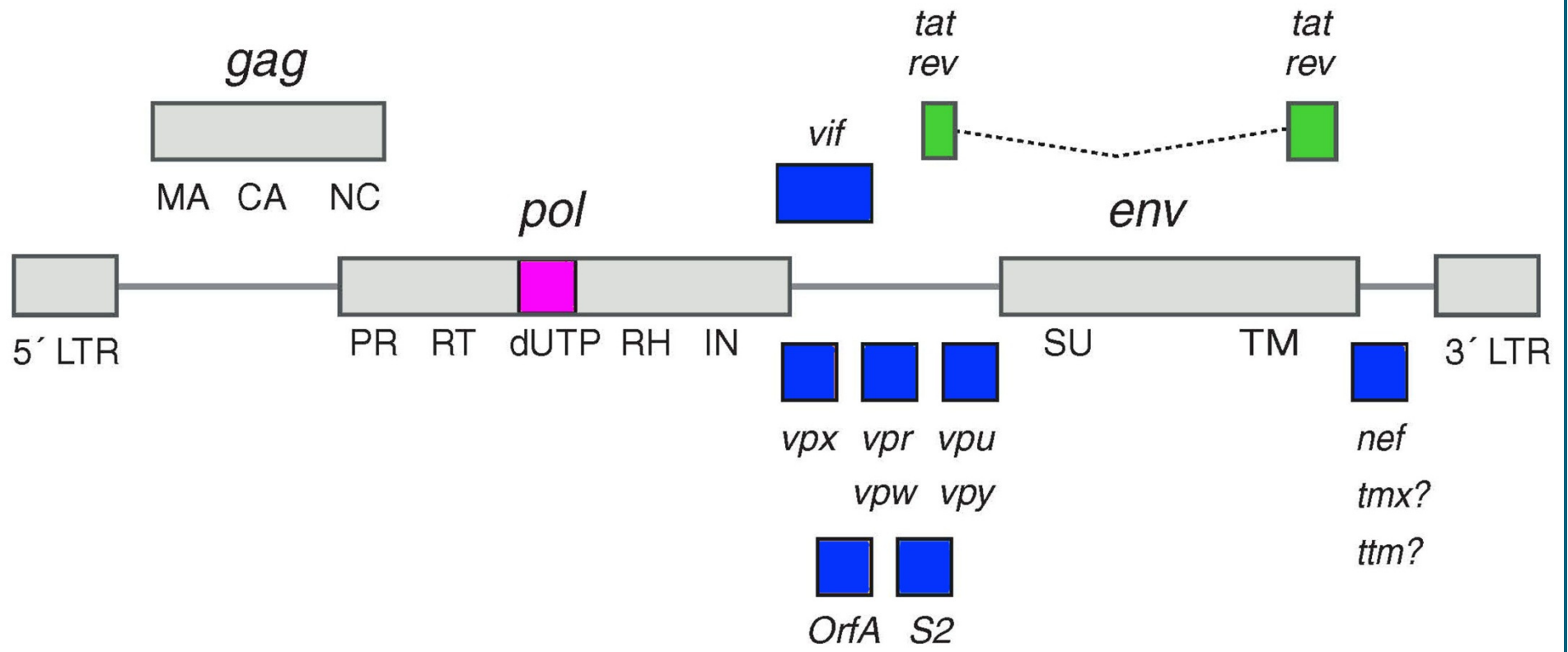
Infect Non-Dividing Cells

Hostile environment

Low dNTP (SAMHD1) High dUTP

Evolved Additional ORFs to GAG, POL, ENV

Complex Retroviruses



Additional / Ancillary Lentiviral Genes

Tat Transactivator for replication

Rev Export of viral RNA from nucleus

dUTPase dUTP → dUMP

S2 Binds cellular proteins
Inflammatory cytokine ↑

Vif APOβEC3 degradation

Vpoc SAMHD1 degradation
APOβEC3 degradation

Vpr DCAF1 G2 arrest

Vpu CD4 ↓
Tetherin ↓

OrfA CD134 ↓

nef CD4–signaling
–intracellular trafficking
–cell migration
–apoptotic pathways

Timeline of Lentiviral Evolution

Molecular Clock Estimate

Entire Genus = < 1 million years

Individual Lentiviruses = 100's to 1000's years

Human	HIV-1M	1908 – 1933
Gorilla	SIVgov	1818 – 1906
Chimpanzee	SIVcp2	1266 – 1685



Wertheim et al., 2009

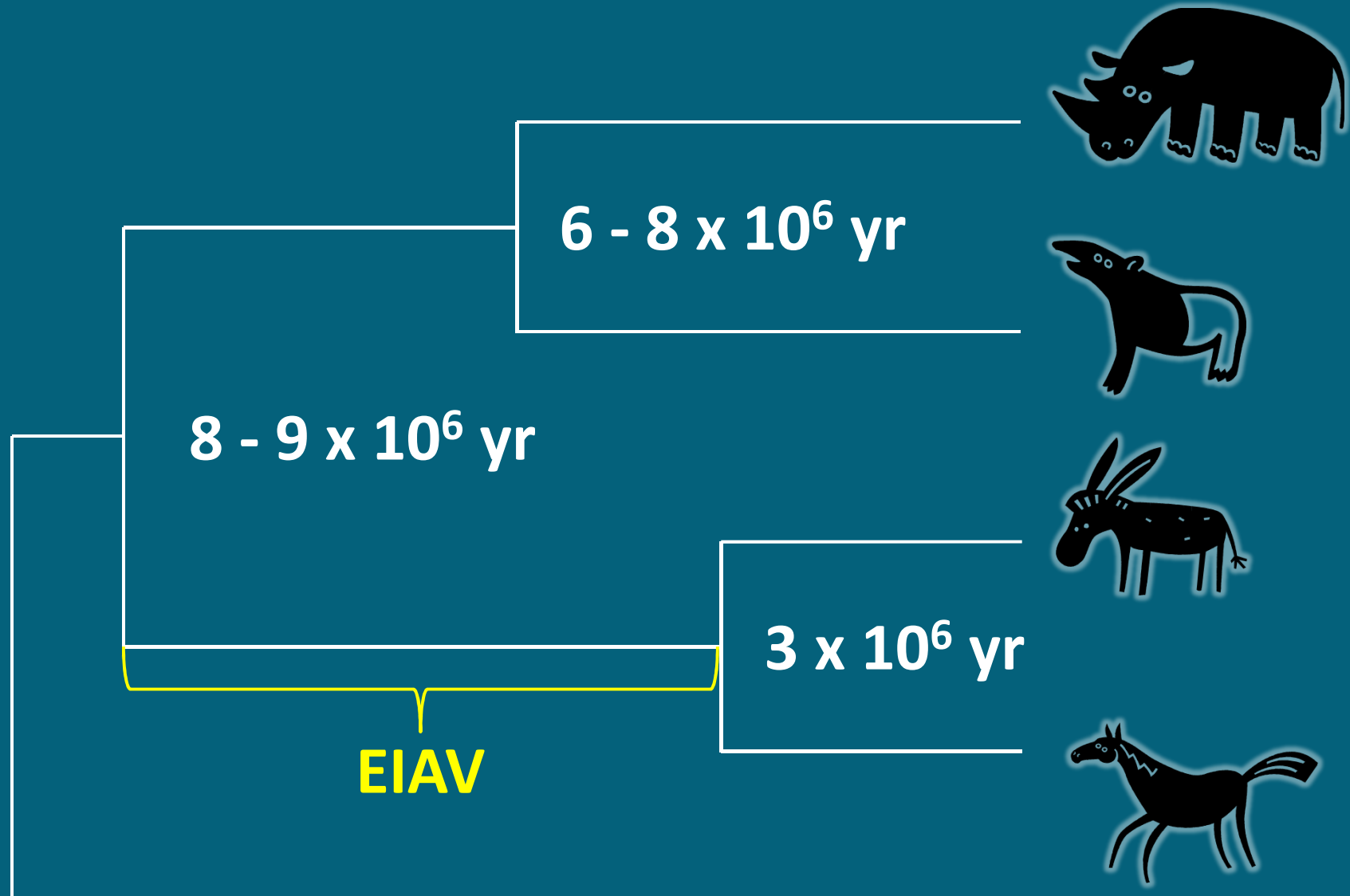
Worobey et al., 2008

Lentivirus Distribution

Retroviruses All Vertebrates



Perissodactyla



Revised Timeline of Lentiviral Evolution

Defective



RELIK

12×10^6 yr

Endogenous



PSIV

4.2×10^6 yr

Lentiviruses



ELVmpf

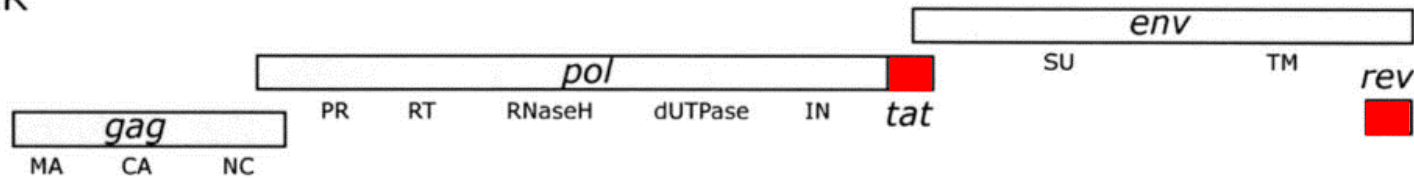
12×10^6 yr

Endogenous Lentiviruses

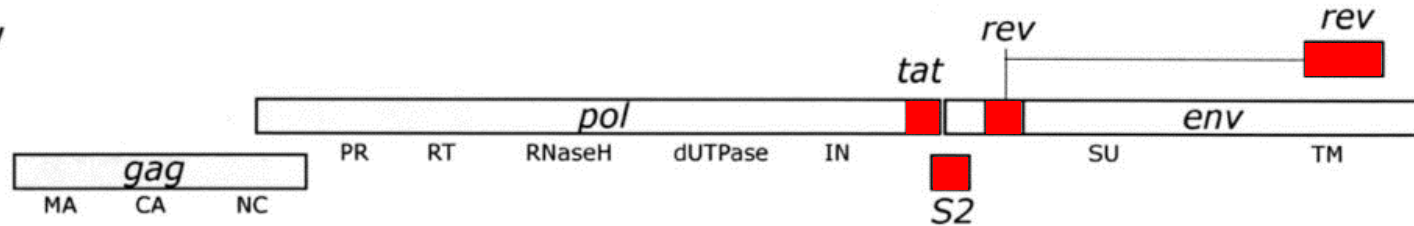
- Older
- Expand Host Range
 - Lagomorph
 - Prosimian
 - Carnivore
- Extinction

Lentiviral Genome Organization

RELIK



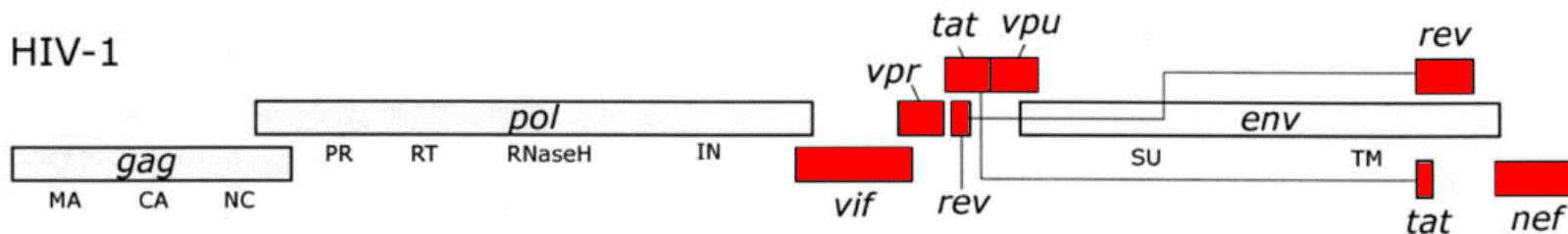
EIAV



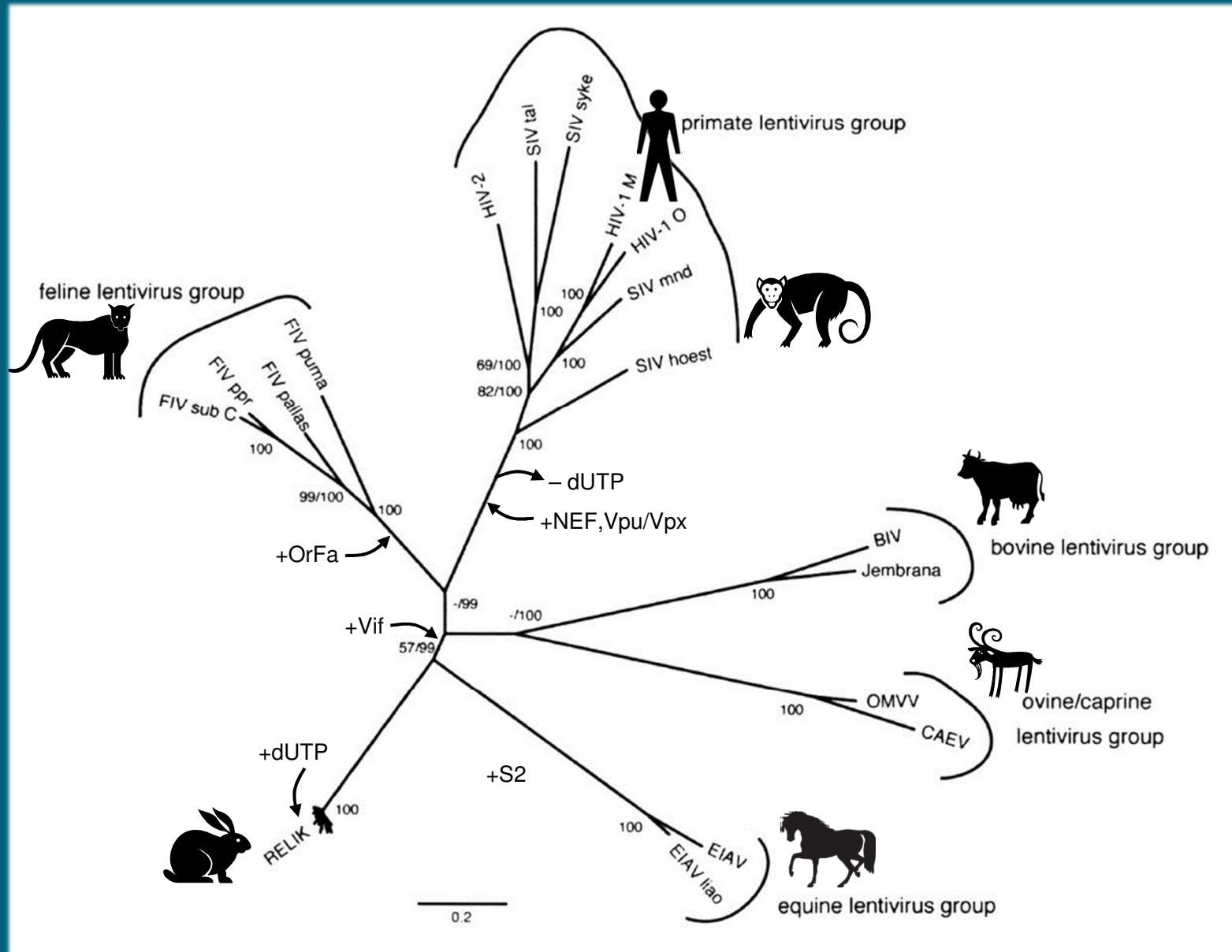
FIV/BIV/OMVV



HIV-1



Lentiviral Phylogeny ?



EIAV

- Infects ALL Equidae
- Persistent Infection – NOT eliminated by host responses
- Simplest genome organization of any extant Lentivirus
 - dUTP
 - TAT
 - REV
 - S2
- Only extant Lentivirus - VIF

EIAV and Retroviral Restriction Factors

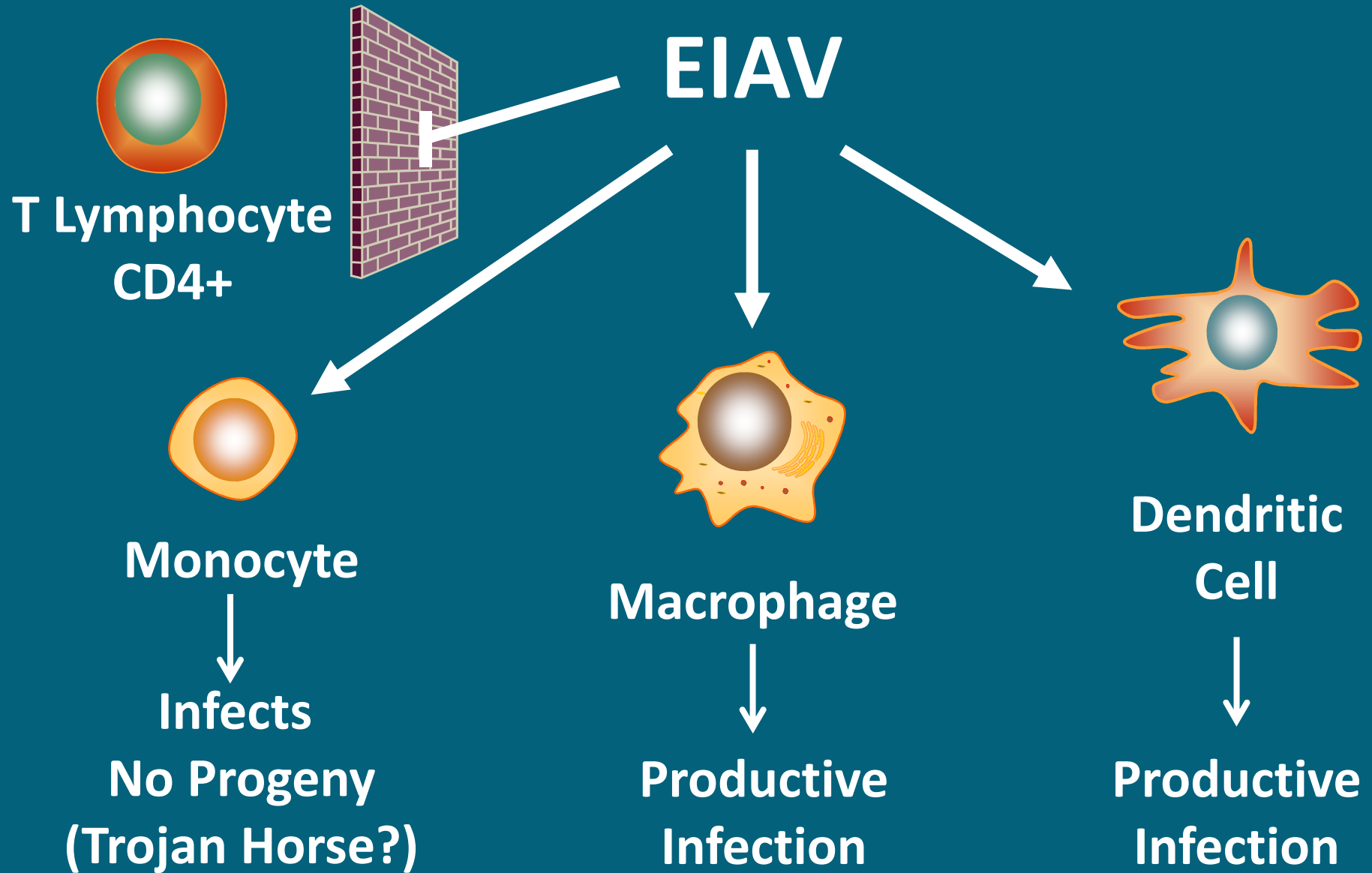
- APO β EC3**
- Horse more genes than any other non-primate species
 - Not blocked by EIAV
 - Packaged in virions
 - ?

- TRIM5 α**
- EIAV p26 resistant?
 - Expression in horses?
(Δ TRIM5 α in Canidae)

- Tetherin**
- EIAV Env resistant?

Is EIAV a primitive lentivirus?

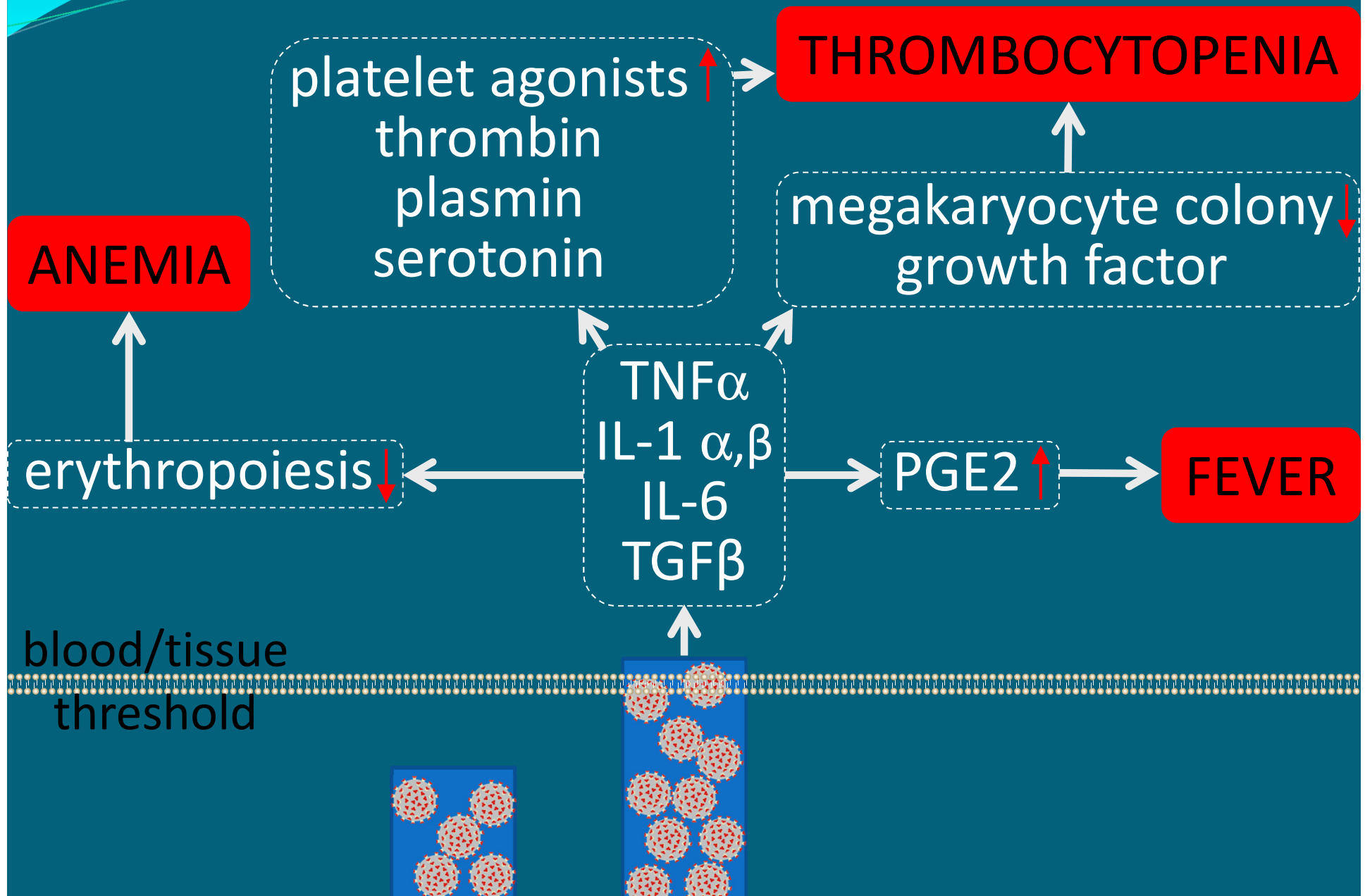
Host Cell Types



EIA Clinical Signs

Stage	Signs	Viral Loads
Acute	Fever (>39°C) Thrombocytopenia Lethargy	High
Chronic (12-24 months)	Fever Thrombocytopenia Petechial hemorrhaging Anemia Edema Cachexia	High
Inapparent	None	Low

Pathogenesis of Acute EIA

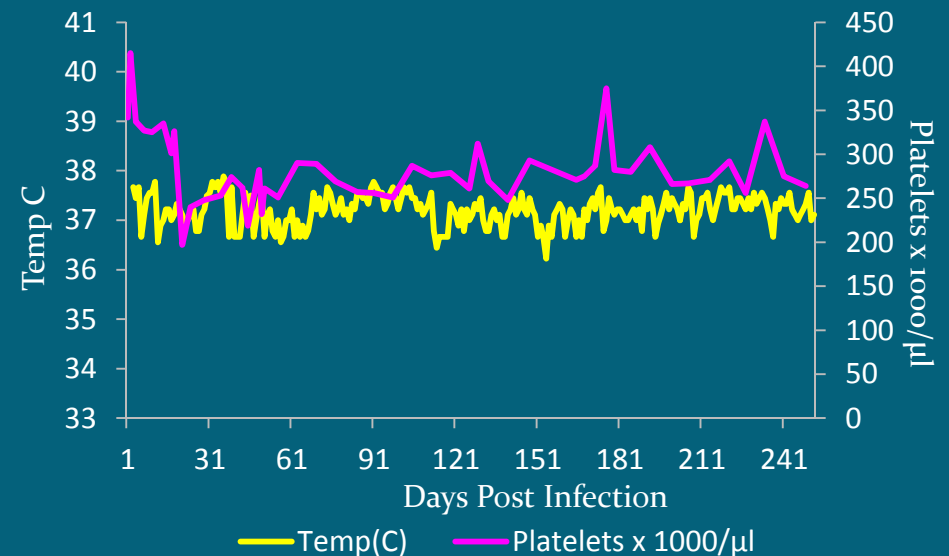
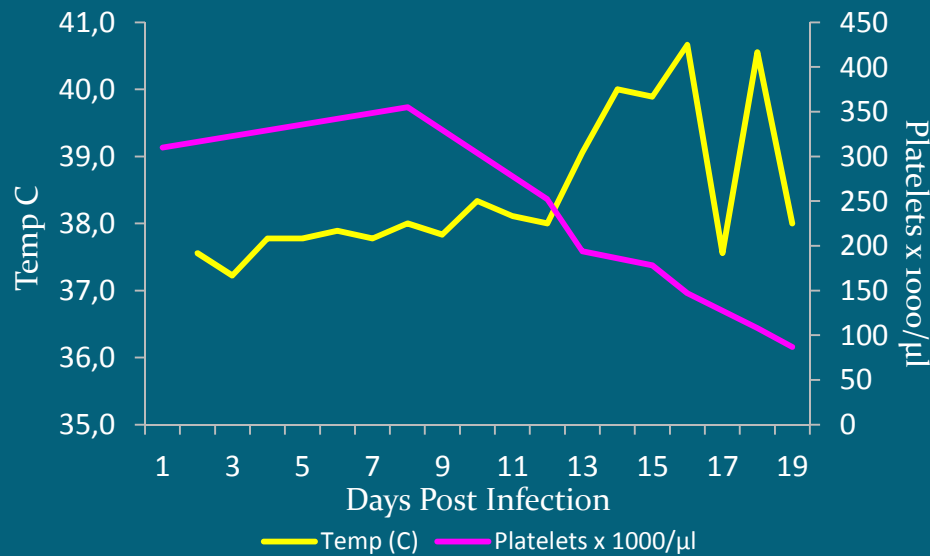


EIA Clinical Signs

Highly variable
Sub-clinical – Death
Individual
Equid Species



EIAV Differences between Equid Species



Host Management of Lentiviral Infections



Natural: SIV / African Non-Human Primates
Subclinical

Viral Replication – High

Immune Control – Ineffective

Limit Pathogenesis, CD4 Depletion

NEF – CD3-TCR↓

Non-Natural: HIV / Humans, SIV / Asian Macaques
AIDS

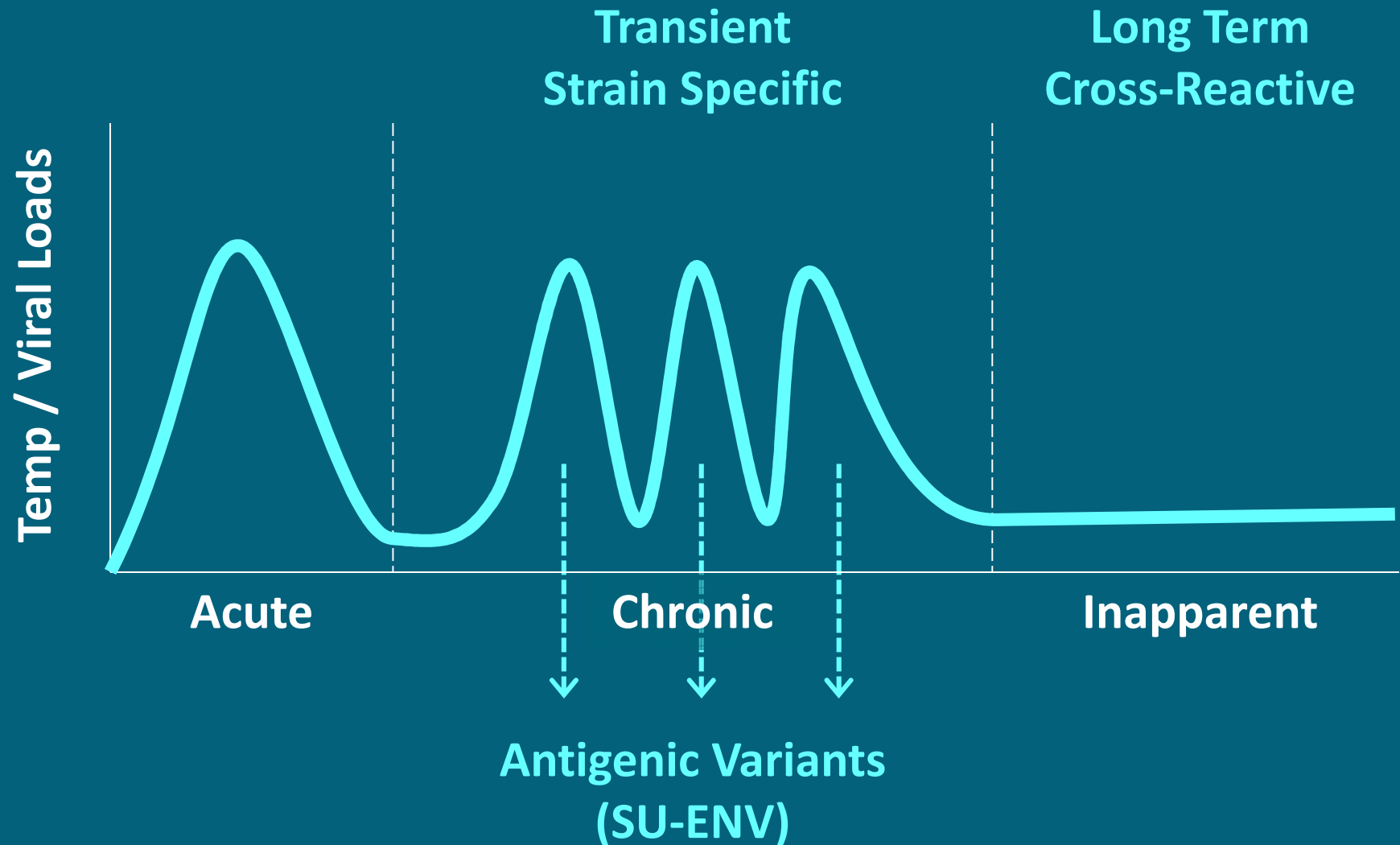
Viral Replication – High

Immune Control – Limited, Transient
(Exception – elite controllers?)

Lack Mechanisms to Limit Pathogenesis

EIAV / Horse

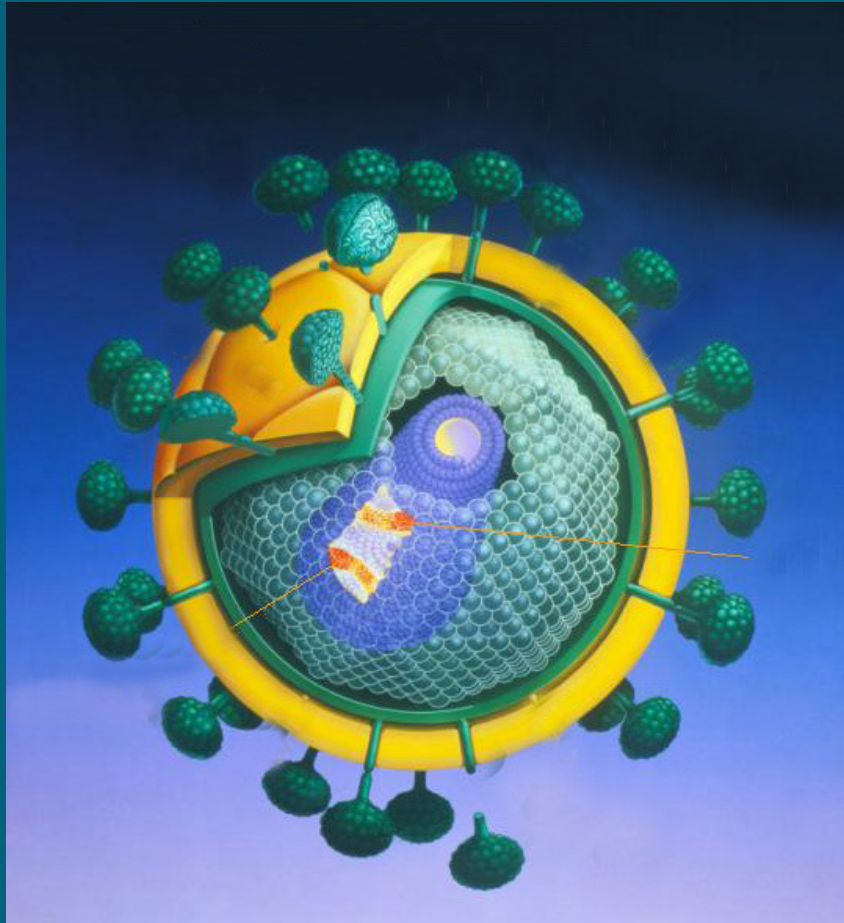
Natural Immune Suppression: Viral Load \uparrow Disease



Immunological Control of EIAV

- No simple correlate of protection
- Differences between individual horses
- Model for Elite Control of HIV in humans?
- Other mechanisms?

EIAV SU : A Critical Role



Attachment/Entry
Neutralizing Epitopes

Variation in SU PND with Time

SU PND Amino Acid Sequence

	N	S	S	D	S	S	N	P	V	R	V	E	D	V	M
I	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
II	•	•	•	•	•	•	•	S	•	•	•	•	•	•	•
II	•	•	G	•	•	•	•	S	•	•	•	•	•	•	•
I															
IV	*	*	*	*	*	*	*	*	*	*	*	*	*	*	•
V	T	•	G	P	•	•	•	S	•	•	G	•	Y	E	T

$\Delta S2$ EIAV Vaccine

Based on EIAV_{UK3} infectious molecular clone (Cook et.al. Virology 313: 588-603, 2003)



EIAV $\Delta S2$

$10^2 - 10^4$

EIAV_{UK3}

$>10^8$

Immunize Horses EIAV $\Delta 2$, challenge >6 months

75-100% Protection from INFECTION
from **HOMOLOGOUS challenge**

EIAV Heterologous Challenge

Pony 564 EIAV_{PV} = EIAV_{UK3} SU = EV0

Febrile episode IV @260dpi (6% divergence = SU = EV6)

Inapparent 1219dpi (13% divergence SU = EV13)



Δ S2 Vaccine Trial

24 Δ S2 Vaccinated Ponies

Challenge

8 EVO
(Homologous Env)

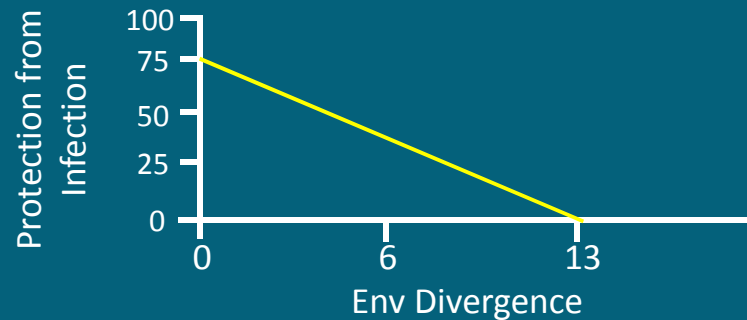
8 EV6
(6% Difference in Env)

8 EV13
(13% Difference in Env)

Protection
Disease 87.5%
Infection 75%

Protection
Disease 62.5%
Infection 37.5%

Protection
Disease 37.5%
Infection 0%



Variation in EIAV SU

- Establishment of persistent infection
- Critical role in vaccine efficacy (related strains)
- Limited by structure and function?

Lesson Learned

- Lentiviruses: complex $>12 \times 10^6$ YR
- Few mammalian hosts – Extinct
- EIAV: Least complex genome
 - Successful/Persistent
 - Integration
 - MØ host cell
 - Neutralization Ab resistant
 - Antigenic variation

Lesson Learned

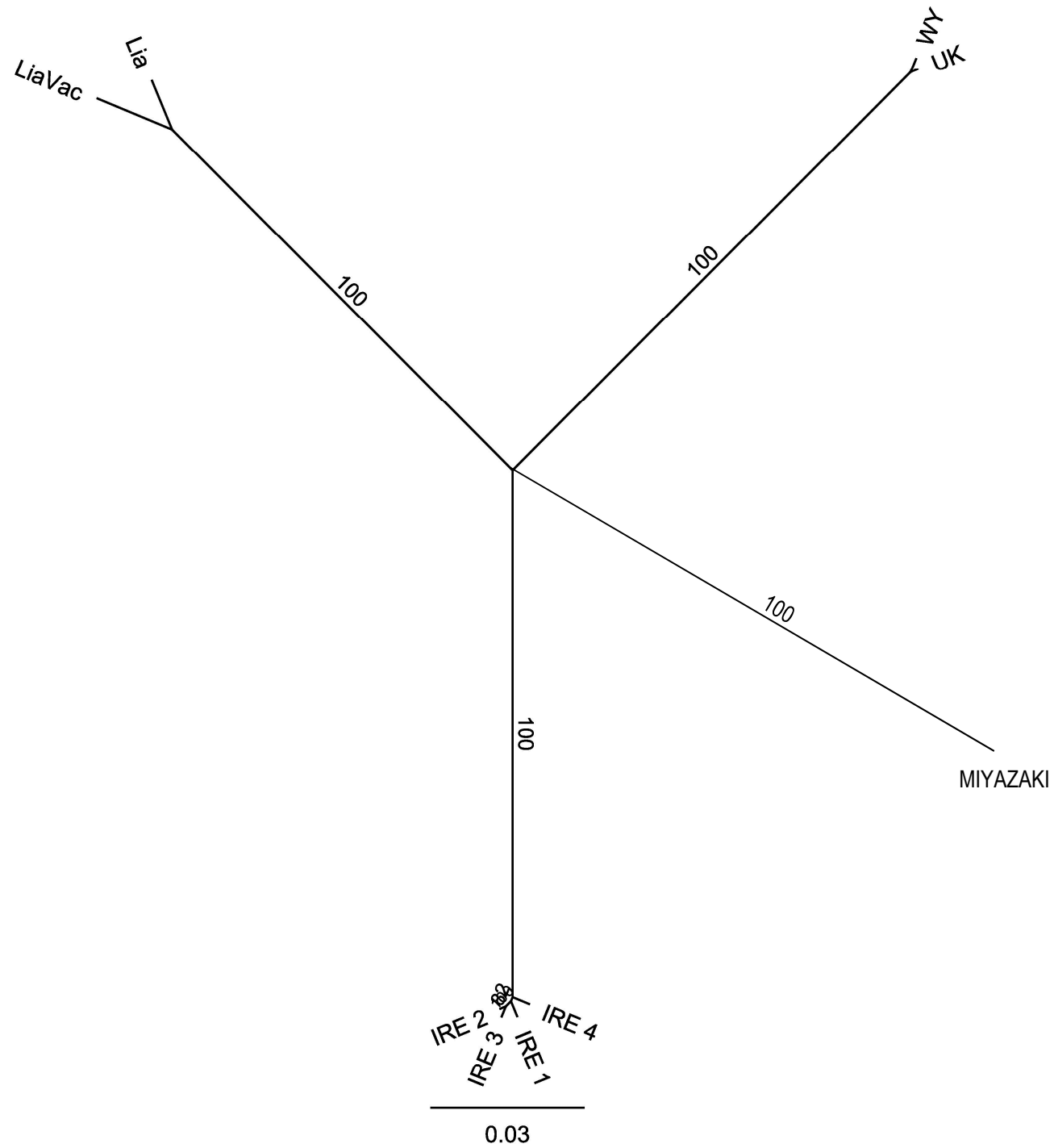
- Disease = High Viral Loads
- Inapparent Carrier = Low Viral Loads
 - Immunological Control
 - Horse = Model for Elite HIV Control?
- Vaccine Efficacy : Variation in SU

Lessons to be Learned

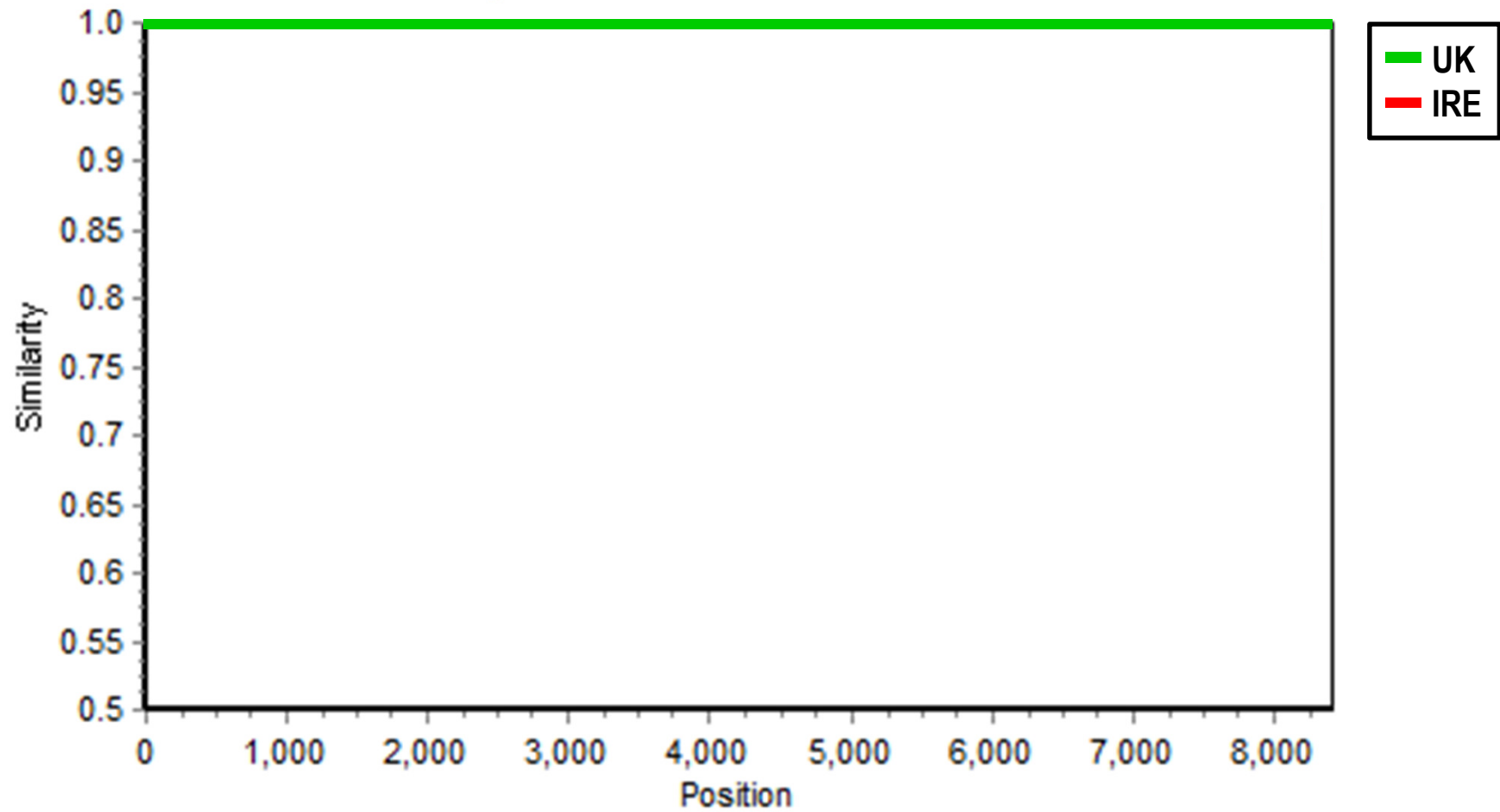
- ? Extent of EIAV Diversity
 - ❖ Molecular Diagnostics
 - ❖ Vaccines
- ? EIAV Persistence Mechanisms
- ? Evasion of Host Restriction factors
 - ❖ NO VIF/VPU

Lessons to be Learned

- Immunological Control Mechanisms
 - ❖ NO Simple correlations
 - ❖ Other mechanisms
- ? Differences between Horses in Disease and Control
- ? Differences between Equid species



SimPlot - Query: AF016316_UK
FileName: D:\My Documents\EIAV IRE\UK vs IRE.fasta



Window: 200 bp, Step: 20 bp, GapStrip: On, Kimura (2-parameter), T/t: 2.0

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