Winter School seminars

speaker	title	session	date	time	GT	TG e Neuro
Wenting Zhao NTU Singapore (online)	Engineered membrane deformation and guided reorganization of cellular machinery to study the mechanical properties of cells	Nuclear Integrity and chromatin organization	18/12/23	10-11.30	Colonnelli Virgilio Jeong Bernardi	
Fred Bernard Jacques Monod Institute, Paris, France	The importance of nucleus positioning in embryo development	Nuclear Integrity and chromatin organization	18/12/23	11.30-13	Colonnelli Hazrati Bastianelli Bernardi	
Barbara Peruzzi IRCCS Children Hospital Bambino Gesù, Rome, Italy	Nuclear lamins and nuclear dysmorphism in pathologies through advanced microscopy lens	Nuclear Integrity and chromatin organization	18/12/23	14.00-15.30	Majaliwa colonnelli Fanelli Pace	
Jeremy Carlton King's College, London, UK	The ESCRT machinery at nuclear envelope: Closing holes and expanding roles	Nuclear Integrity and chromatin organization	18/12/23	15.30-17	Colonnelli Pace Fanelli Jeong	
Philippe Collas University of Oslo, Norway	D matters: epigenetic and chromatin conformation changes at multiple scales during adipocyte differentiation	Chromatin organization in development	19/12/23	10.00-11.30	Virgilio Antonio	
Fulvio Chiacchiera, University of Trento	Role of chromatin-associated complexes during liver regeneration and tumor formation	Chromatin organization in development	19/12/23	11.30-13.00		

Winter School seminars

Daniela Palacios Cattolica University of Rome	Epitranscriptomics in muscle development and disease	Chromatin organization in development	19/12/23	14.00-15.30	Pace Bartolini Pourali	
Matthieu Boulard European Molecular Biology Laboratory EMBL, Rome	Sweet gene regulation in the mammalian embryo	Chromatin organization in development	19/12/23	15.30-17.00	Majaliwa Antonio Pourali Bastianelli Hazrati Bartolini Pace	
Jamie Hackett European Molecular Biology Laboratory EMBL, Rome	What do chromatin modifications do? Epigenome editing to dissect function in health and disease?	Genome Stability in mouse model of diseases	20/12/23	10.00-11.30	Hazrati Antonio Pourali Ilie	
Monica Ballarino Sapienza University of Rome	Myogenesis and long non-coding RNAs: a chromatin affair	Genome Stability in mouse model of diseases	20/12/23	11.30-13.00	Fanelli Virgilio Ilie	
Marina Vietri University of Oslo, Norway	Nuclear envelope dynamics at ruptured micronuclei	Genome Stability in mouse model of diseases	20/12/23	14.00-15.30	Jeong Bernardi Hazrati Bartolini	
Rafal Czapiewski University of Edinburgh, UK (online)	Nuclear envelope controls genetic spacetime - focus on genome organization and function.	Genome Stability in mouse model of diseases	20/12/23	15.30-17.00	Majaliwa Antonio Pourali Bartolini Bernardi	

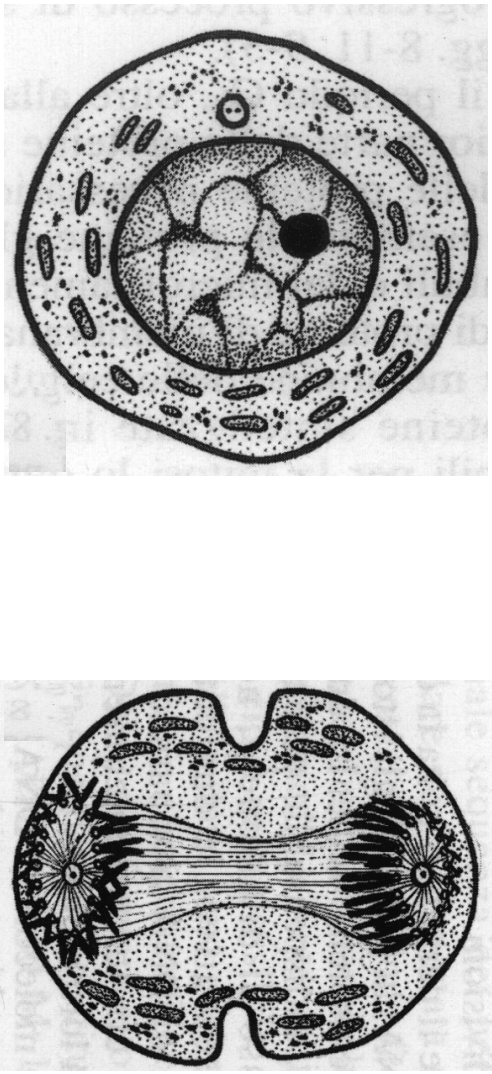
Lentivirus

- Complex genome:
  - Structural genes *gag pol env*
  - Regulatory genes *tat rev*
  - Accessory genes *nef vpr vpu vif (viral fitness)*
- Tropism for macrophages and lymphocytes
- Envelope: inside genome (2 RNA+) + RT, IN, PT
- Persistent infection /progressive chronic disease

Nuclear Translocation Pathway of Viral Genome

Lentivirus
(HIV-1)

Oncoretrovirus



Nuclear transport dependent

- Int (GagMatrix, Vpr)
- cPPT-DNA flap

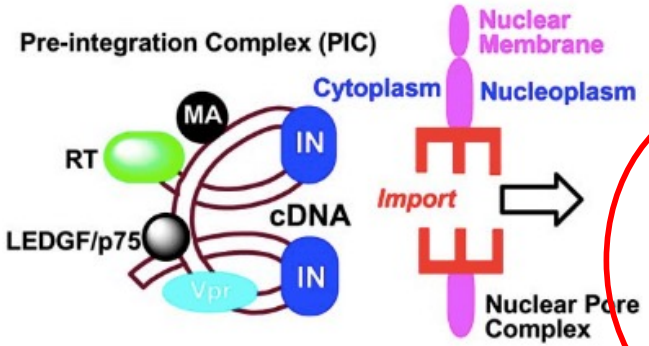
Mitosis-dependent

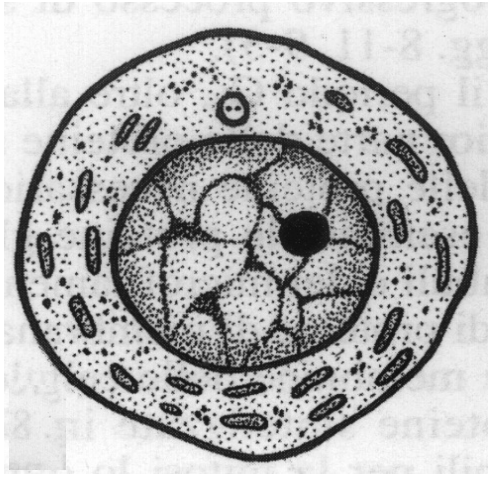
•**cPPT/CTS:** A *central polypurine tract/central termination sequence*

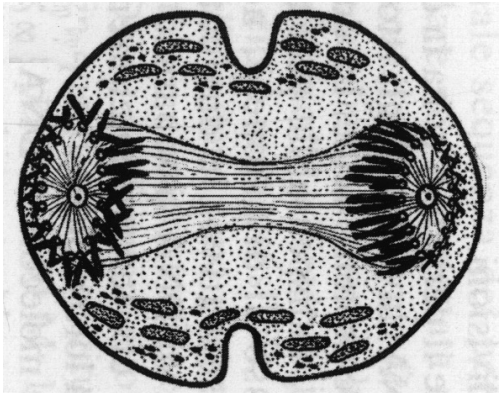
Nuclear Translocation Pathway of Viral Genome

Lentivirus
(HIV-1)

Oncoretrovirus







Mitosis-dependent

•**cPPT/CTS:** A *central polypurine tract/central termination sequence*

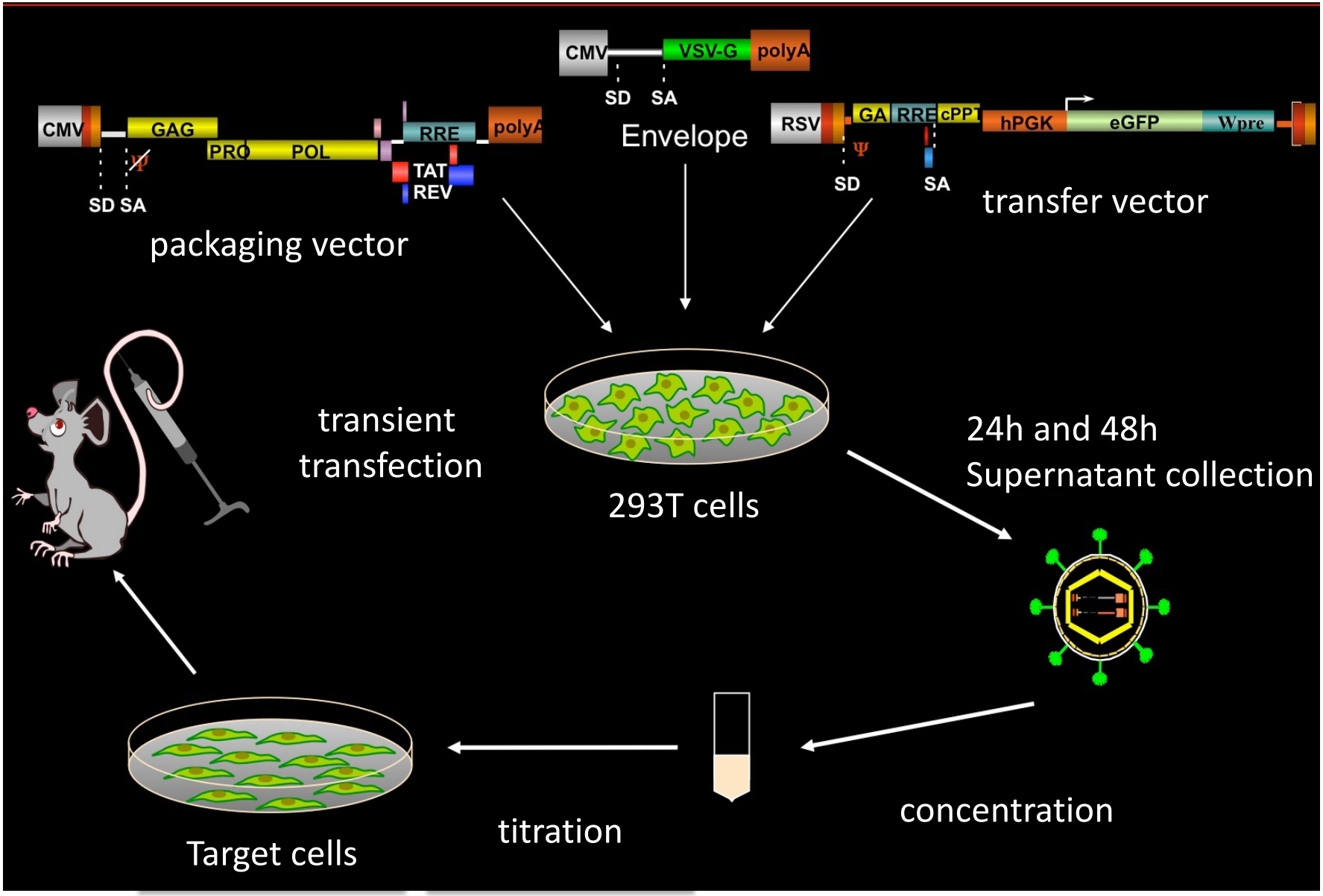
Lentivectors

- *Hybrid viral particles replication defective composed by:*
  - Minimal set HIV1 core proteins
  - Pericapsidic uncorrelated protein(VSV or MLV)
  - Genome containing:
    - transgene expression cassette
    - flanked by HIV1 cis regulatory elements
    - no viral genes

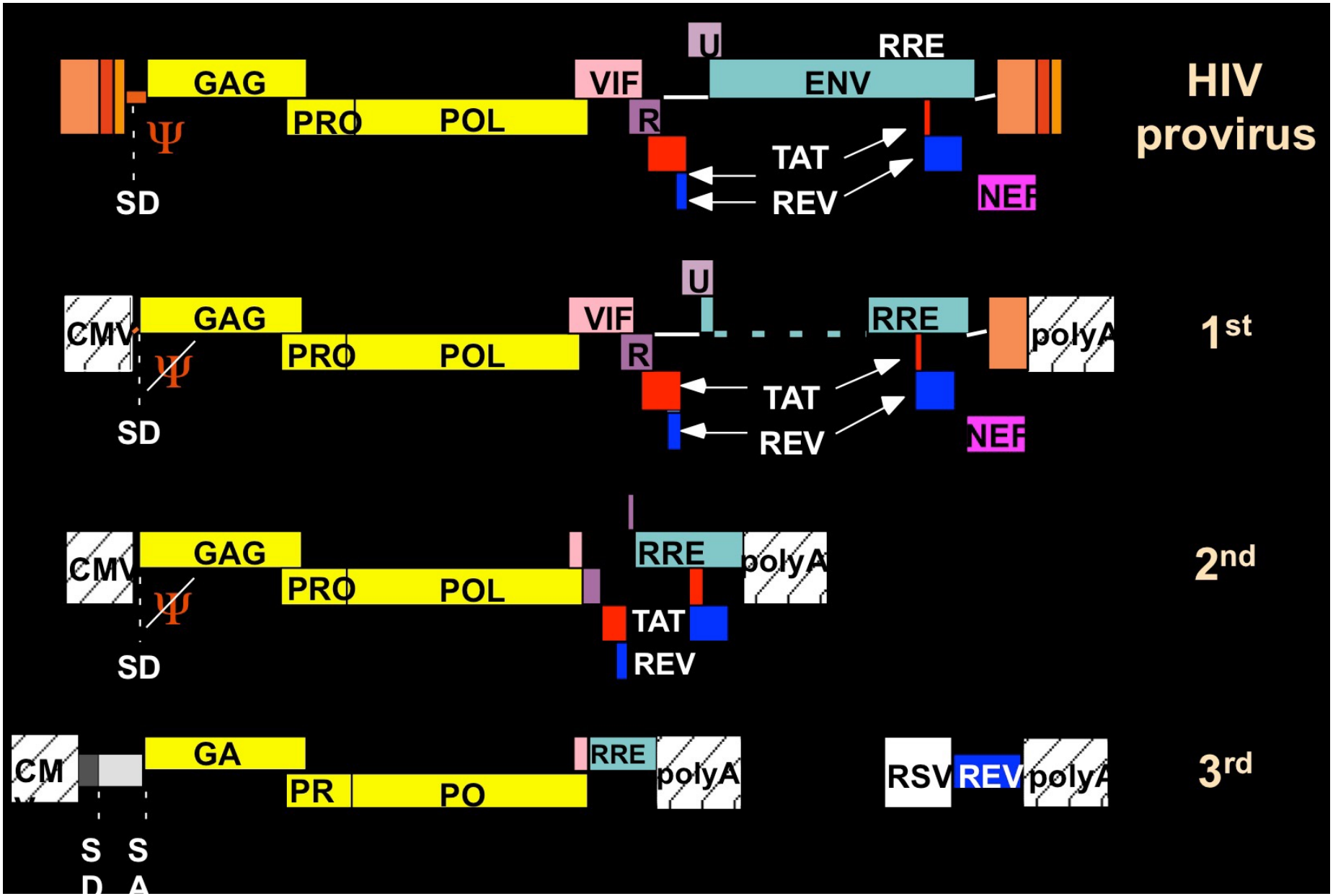
Pathogenesis vs vector biosafety

- tropism for T lymphocytes and macrophages
  - *env* gene DELETED in 1st GEN
- active replication rate and viral load
  - LTR and *tat* gene DELETED in 3rd GEN
  - *nef* gene
  - *vif*, *vpu* genes DELETED in 2nd GEN
  - *vpr* gene
- persistence
- genome diversity

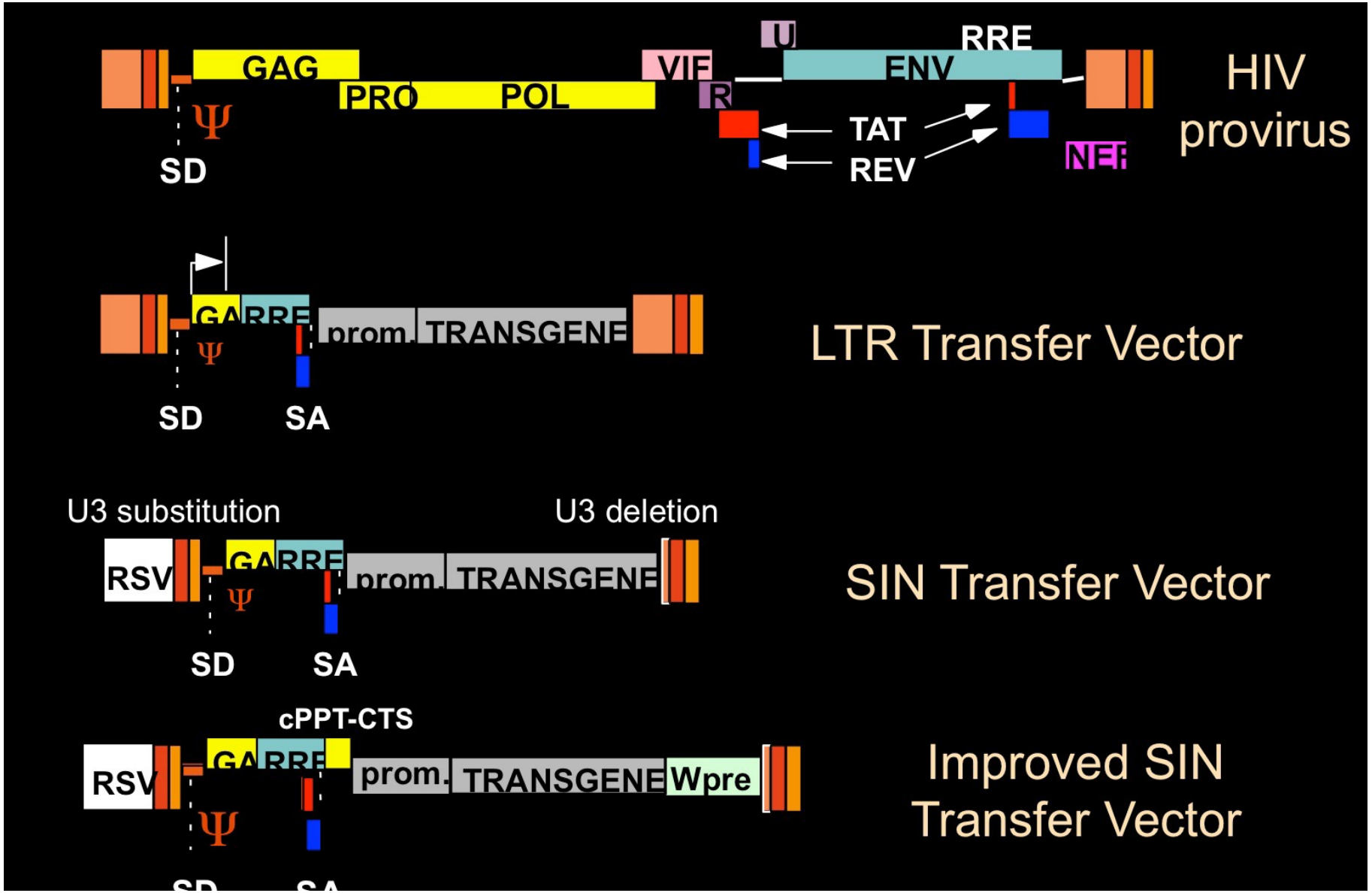
Vector production



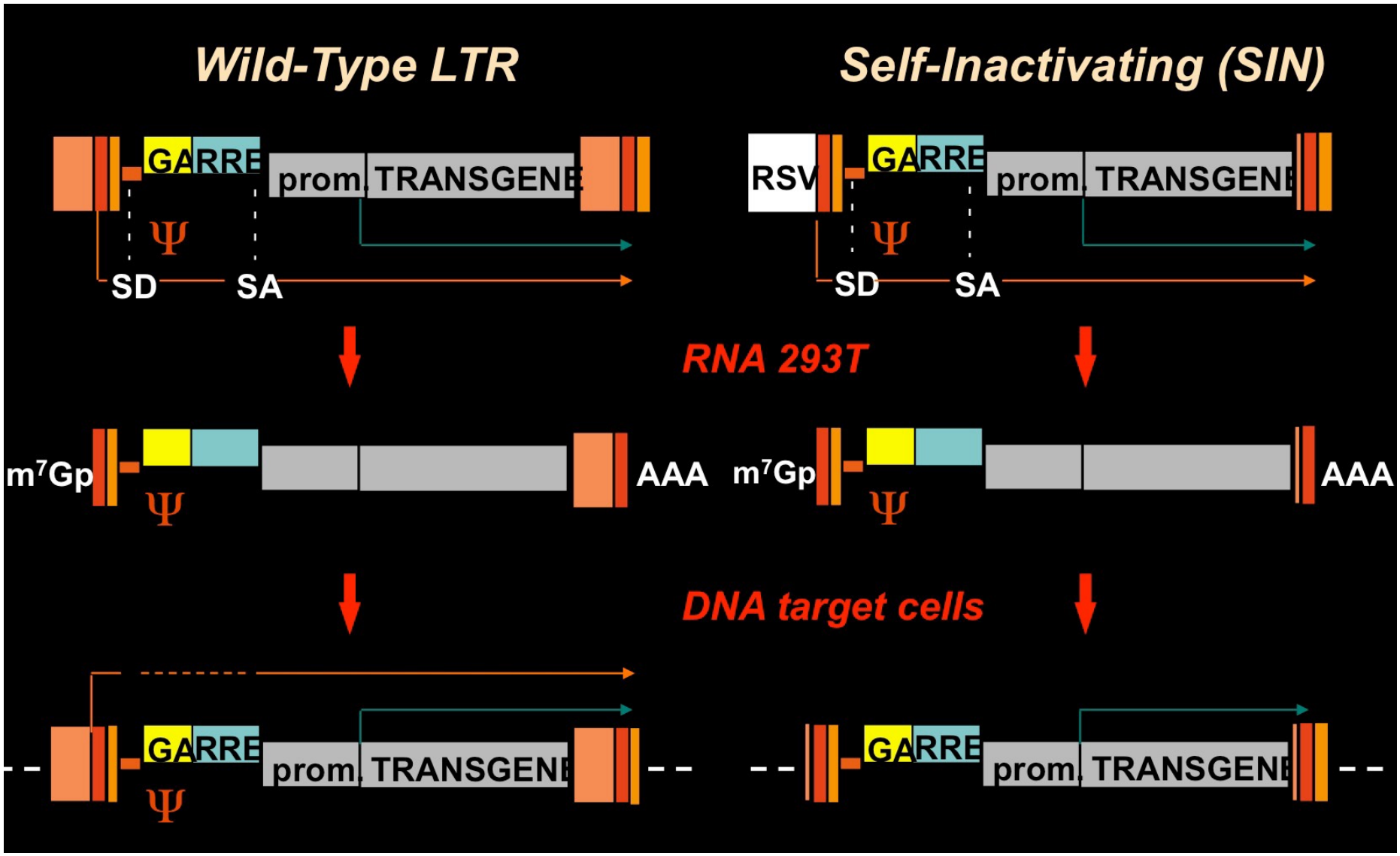
Lentiviral Packaging Constructs



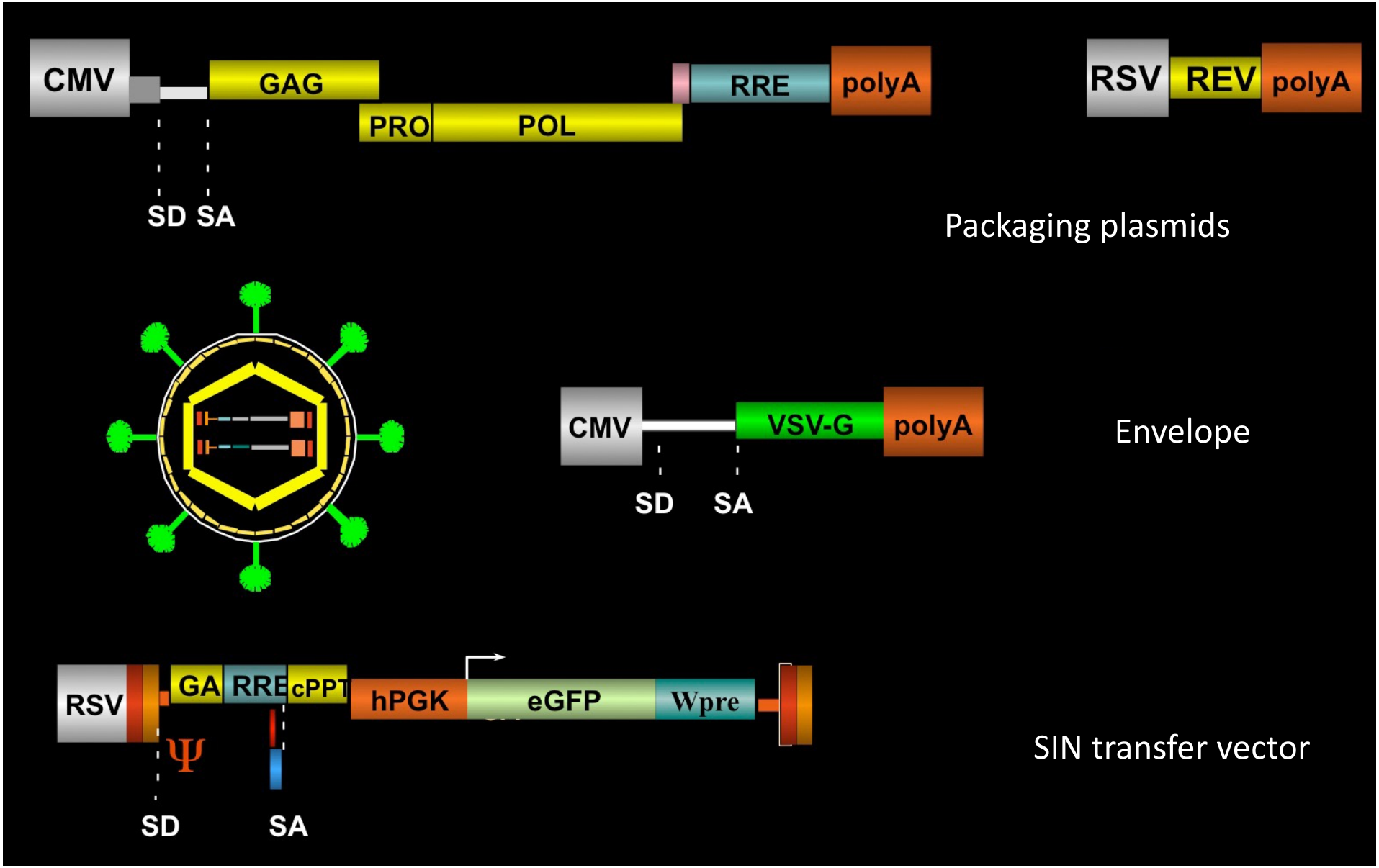
Lentiviral Transfer Vectors



Lentiviral Transfer Vectors



Last (3rd) generation LV

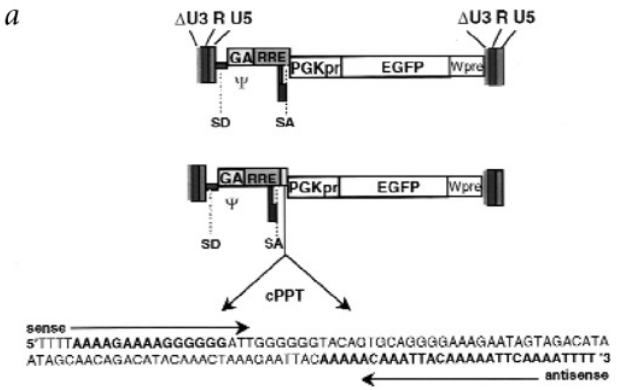


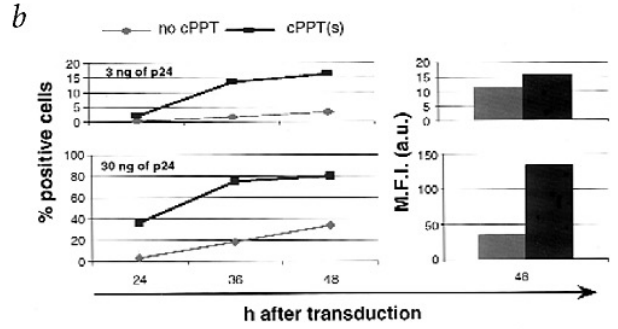
Follenzi et al Nat Genetics 2000
Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences *(on the role of ppt sequence)*

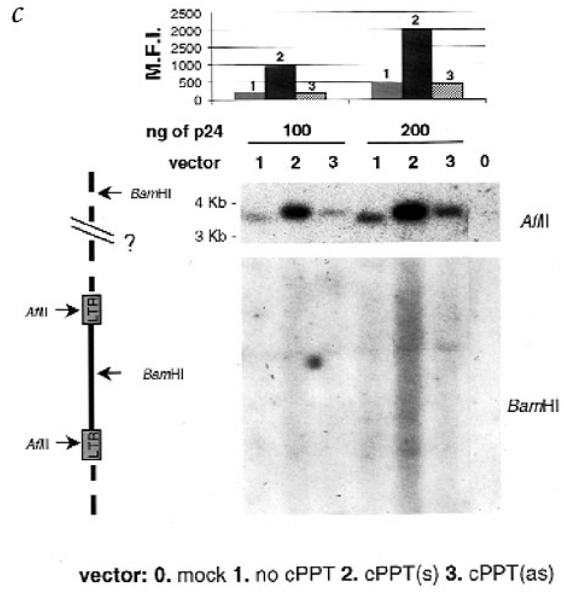
ppt 4-5fold higher titers
10e5TU/ng of p24

Moi and mfi

At high moi
High mfi
Because high integration





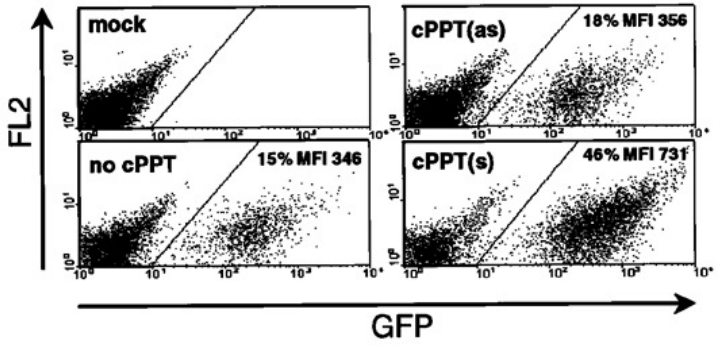


GFP

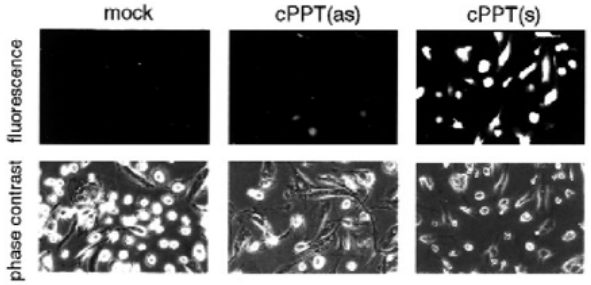
Integrated vector

Improvement in transduction - Primary cultures

a lymphocytes



b macrophages

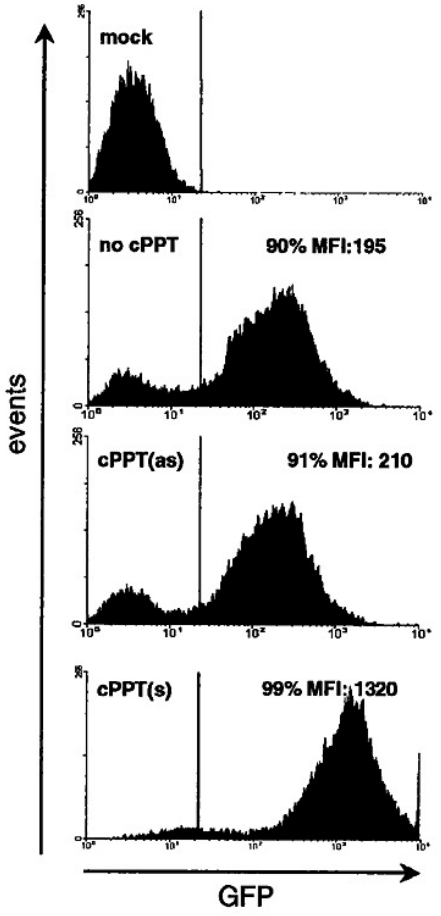


c

Fibroblasts

	growing		quiescent	
	% GFP+	MFI	%GFP+	MFI
no cPPT	76	601	77	134
cPPT(as)	72	654	64	118
cPPT(s)	88	2337	97	1780

d endothelial cells

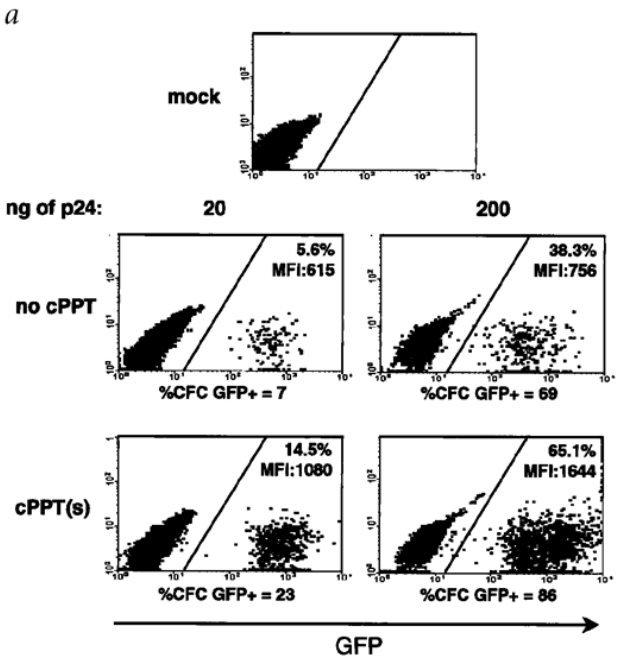


CD34+ stem cells from cord blood

CD34+ stem cells from cord blood
Cytokine independence

CD34+ stem cells from cord blood engrafted in nod-scid mice
Analysis of bone marrow after 3 months, high % of GFP in # cell lineages

a

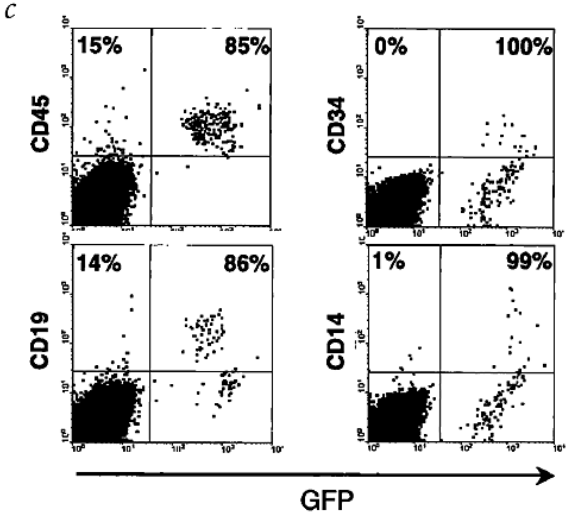


b

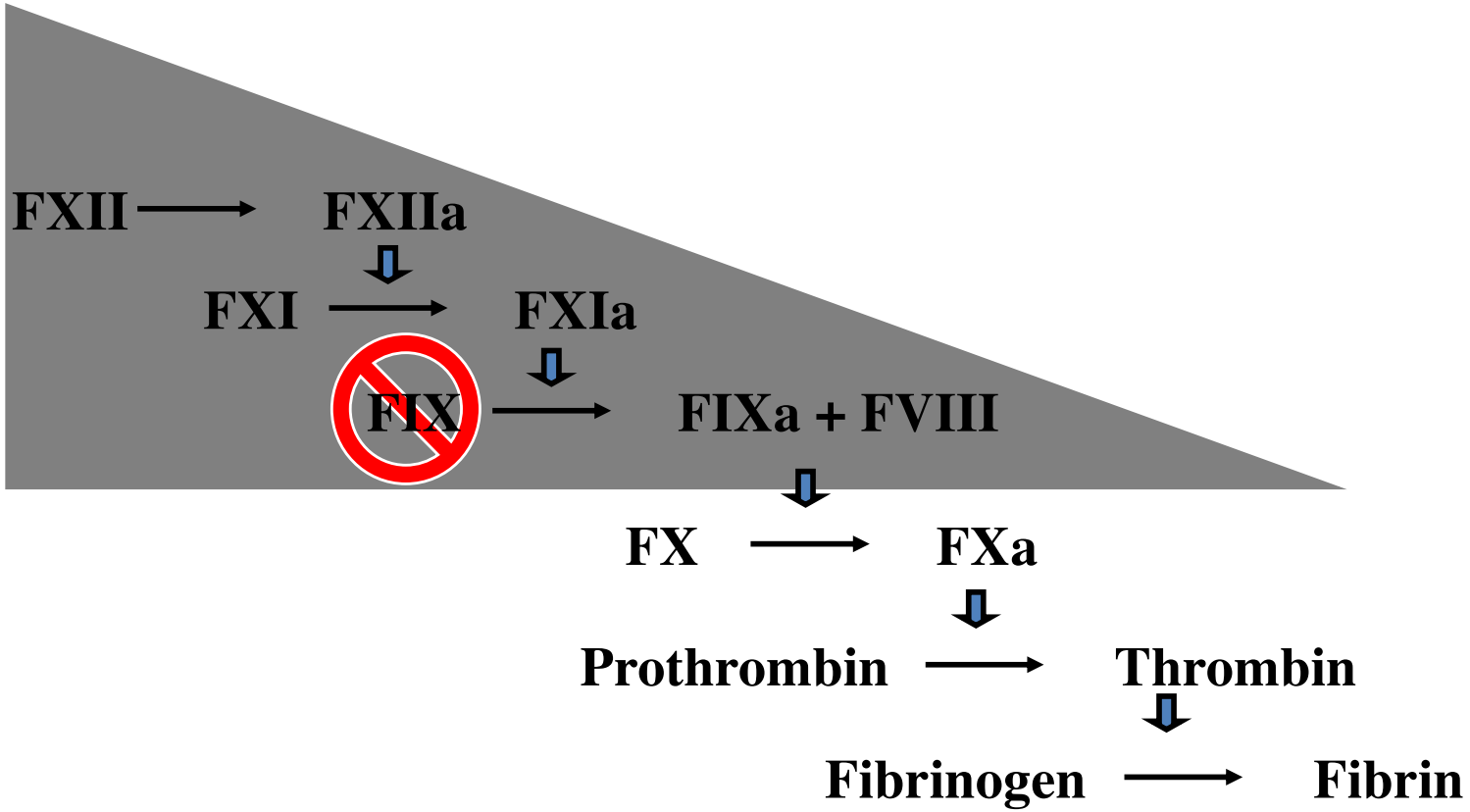
Exp't	cytokines*	%cells GFP+	%CFC CFP+
1	+	87	89
	–	31	79
2	+	79	89
	–	55	84
3	+	65	75
	–	39	70

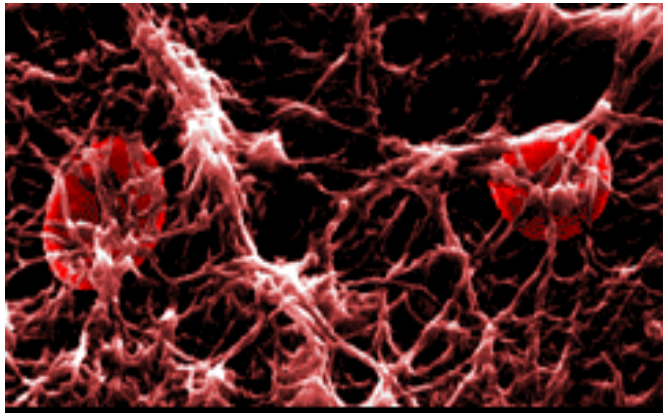
*20 ng/ml IL-3, 20 ng/ml IL-6, 100 ng/ml stem cell factor

c

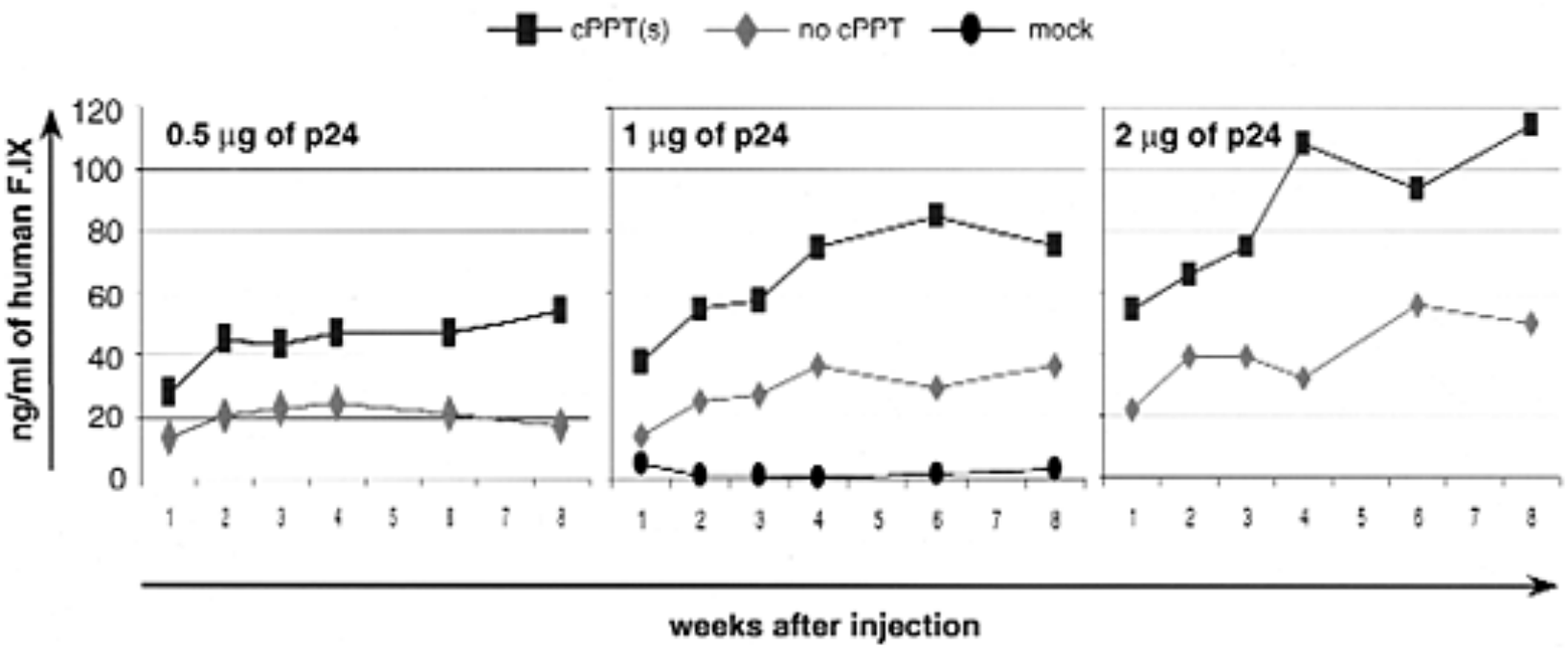


The Intrinsic Pathway of Clotting





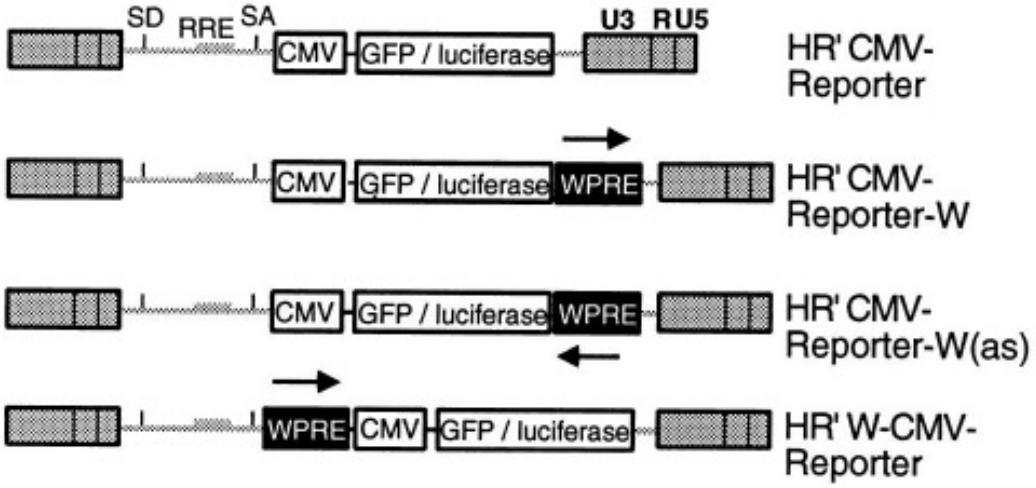
CMV-FIX in tail vein of SCID

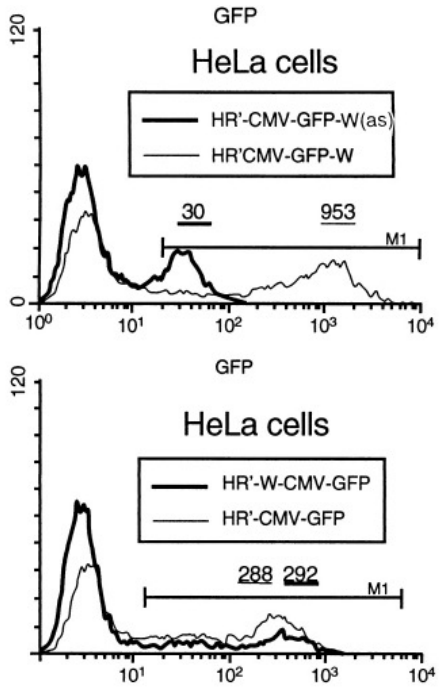


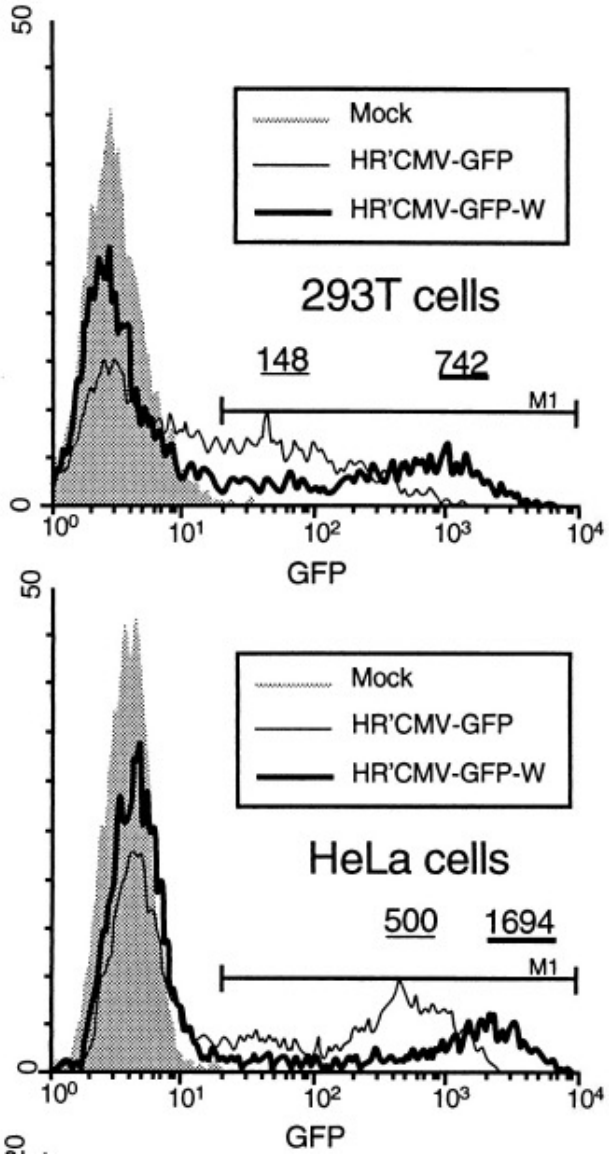
cPPT a *CIS*-acting sequence

- *Restoring cis-acting sequences from HIV-1 pol gene into transfer vector*
  - –promoted
    - nuclear translocation of vector genome
    - increased content of vector DNA in target cells
  - *Nuclear translocation of viral genome is a rate limiting step of infection*
    - – both in dividing and non-dividing cells
    - – dependent on
      - viral proteins
      - *cis*-acting nucleic acid sequences
  - *Restoring nuclear translocation improves vector performance*
    - – increasing particle infectivity
    - – decreasing effective vector dose

WPRE a regulatory sequence enhances transgene expression



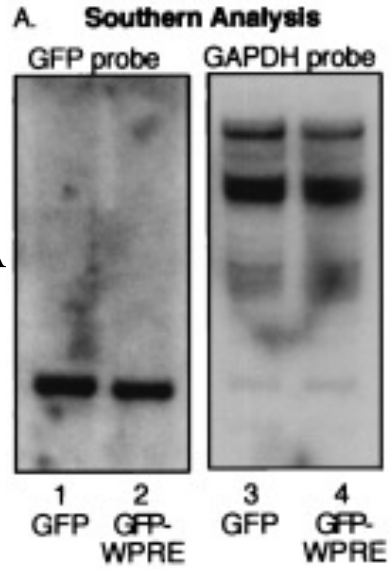




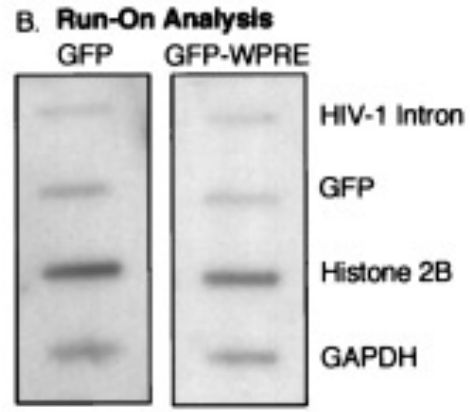
Zufferey R et al, Journal of Virology, 1999

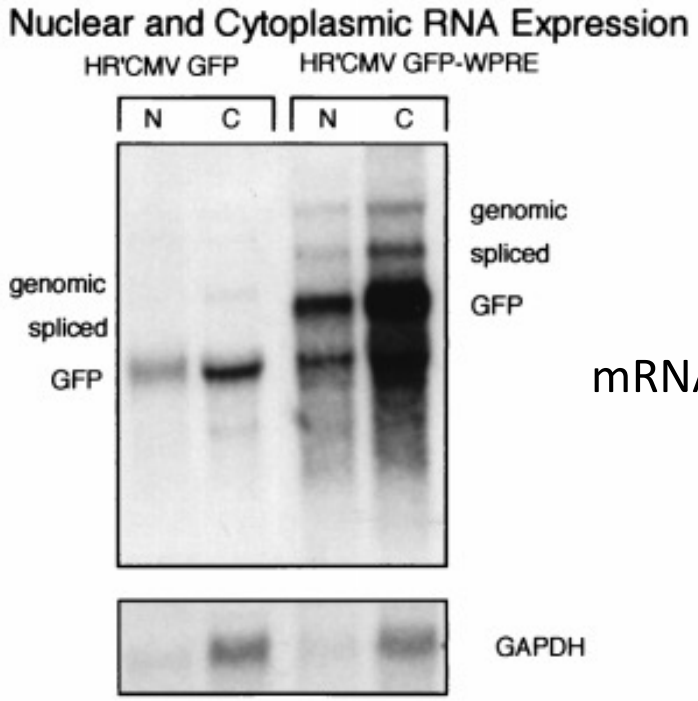
WPRE a regulatory sequence enhances transgene expression

Genomic DNA

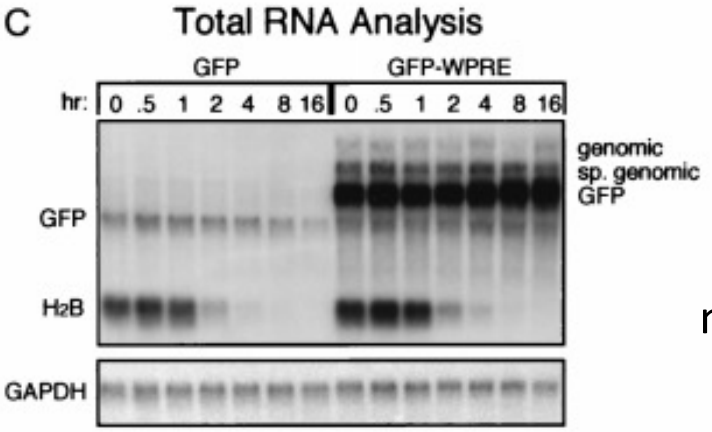


Transcription





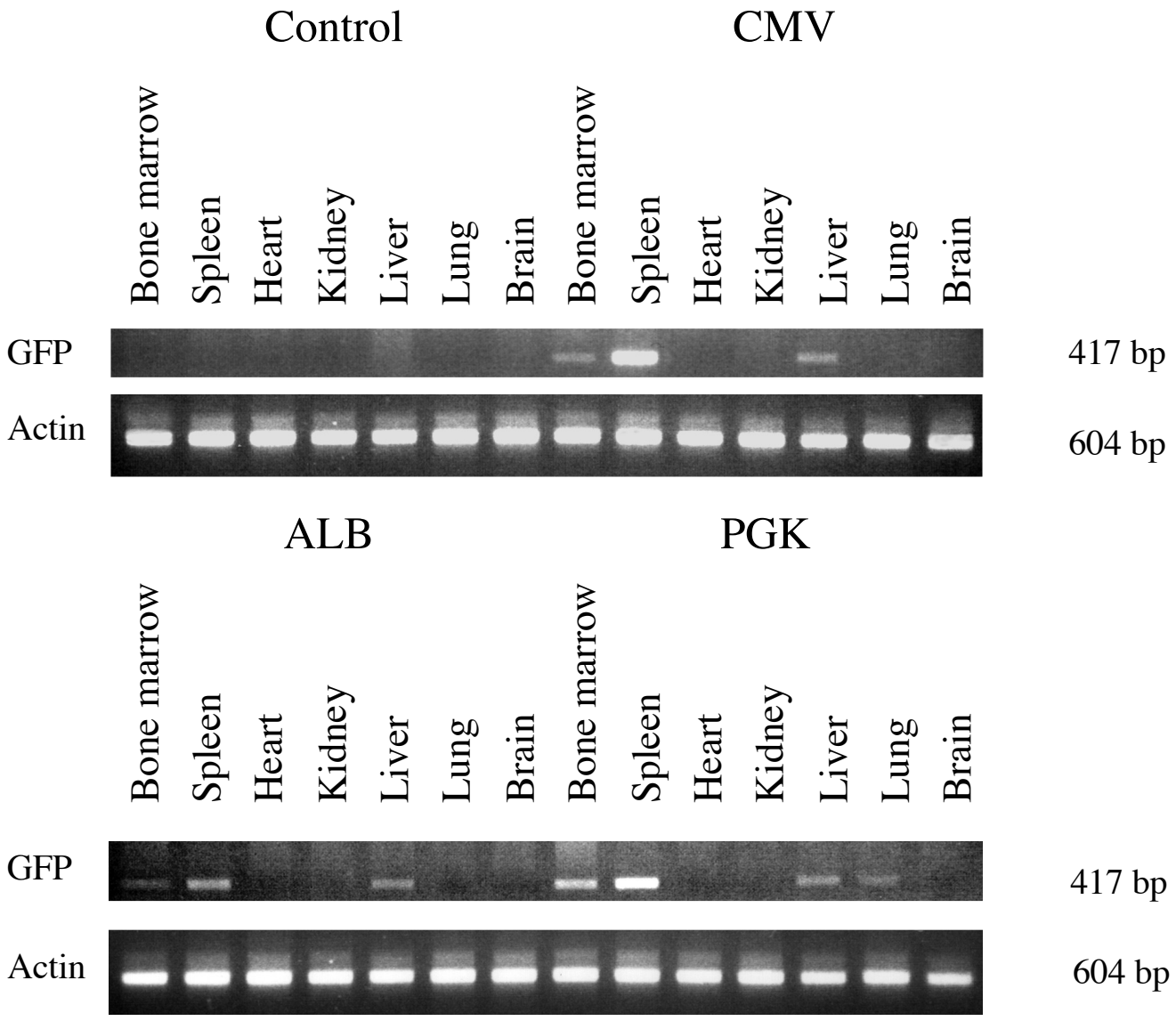
mRNA amount



mRNA stability

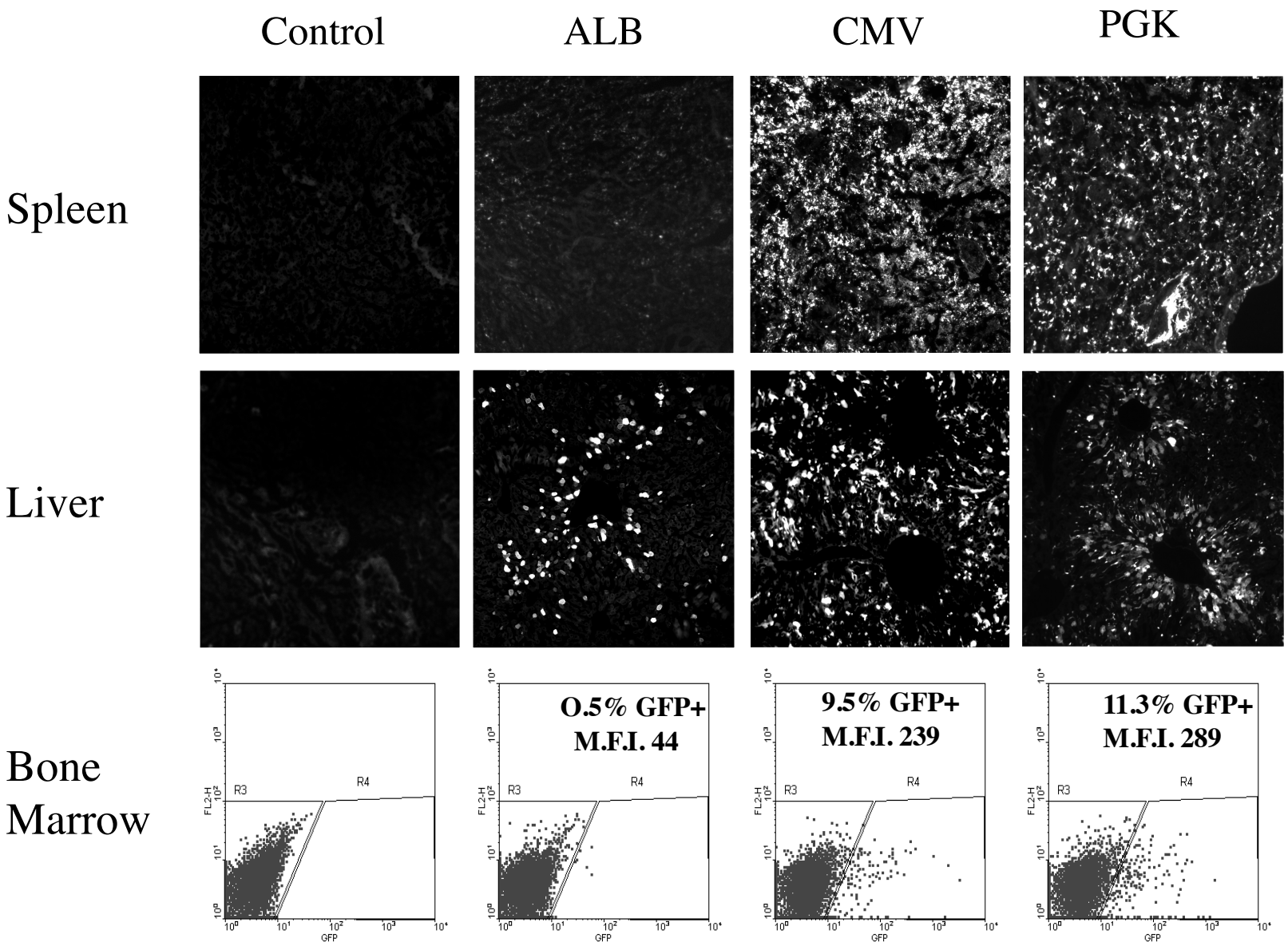
Zufferey R et al, Journal of Virology, 1999

Vector Biodistribution after Systemic Delivery



1 month after vector delivery

Transgene Expression after Systemic Delivery of LV



QUESTIONS?