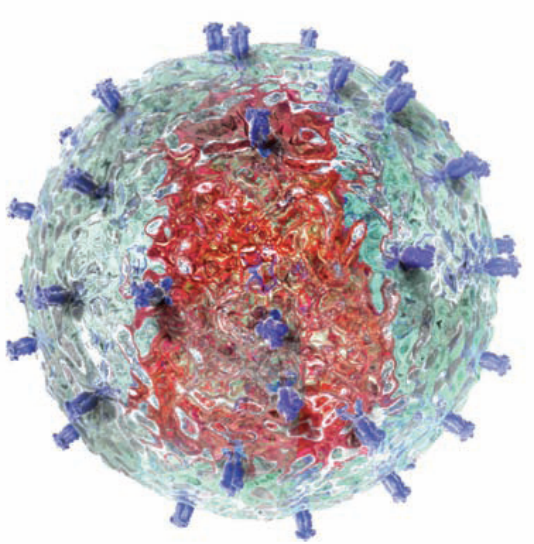
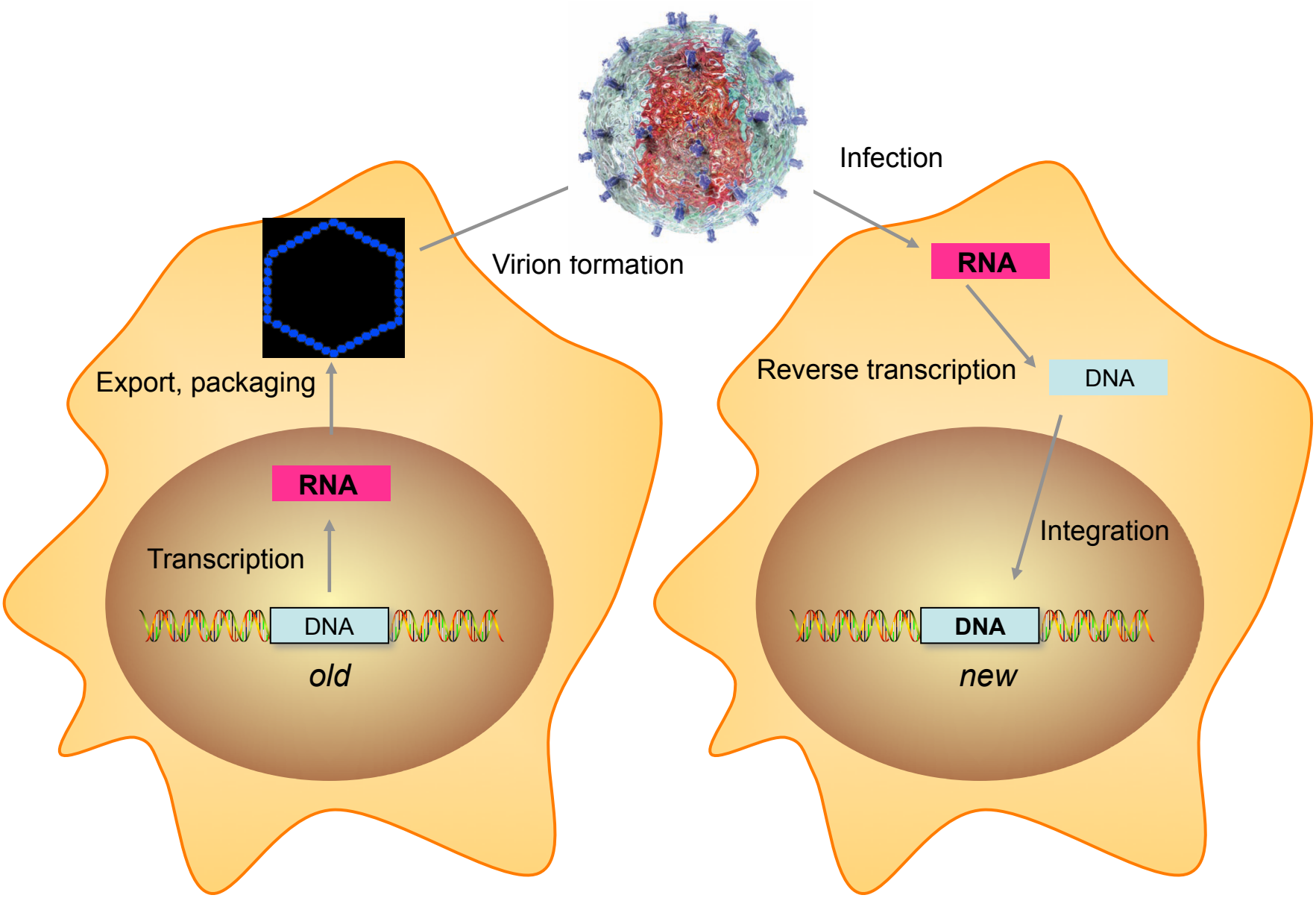


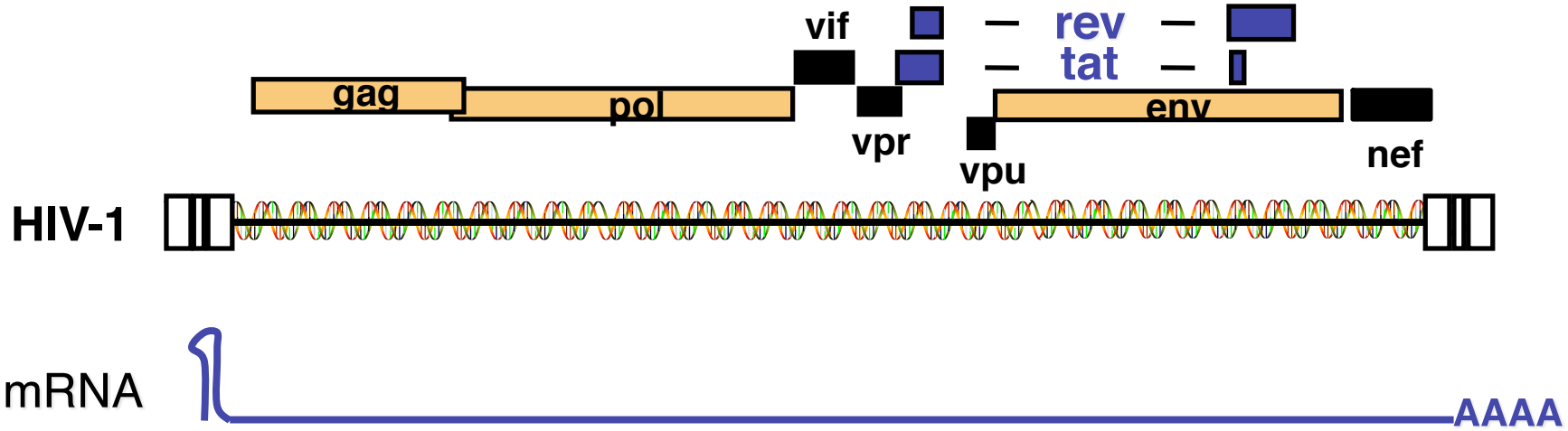
HIV/AIDS: Molecular Biology and pathogenesis

George N. Pavlakis

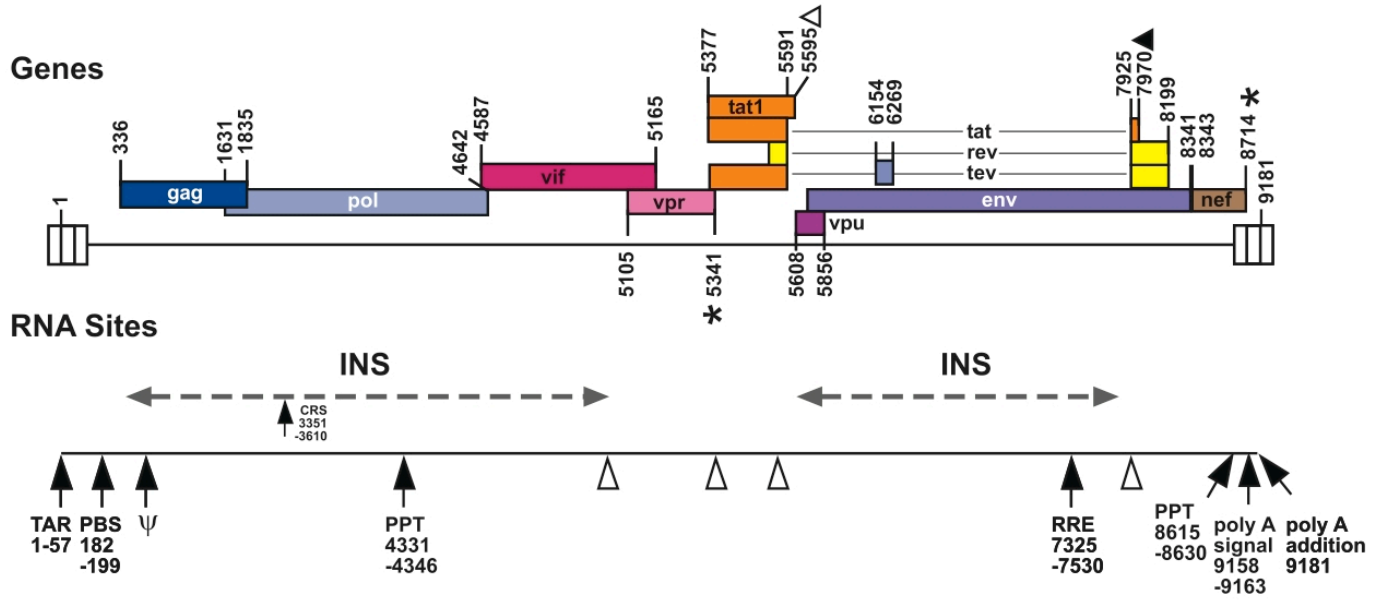
National Cancer Institute, USA

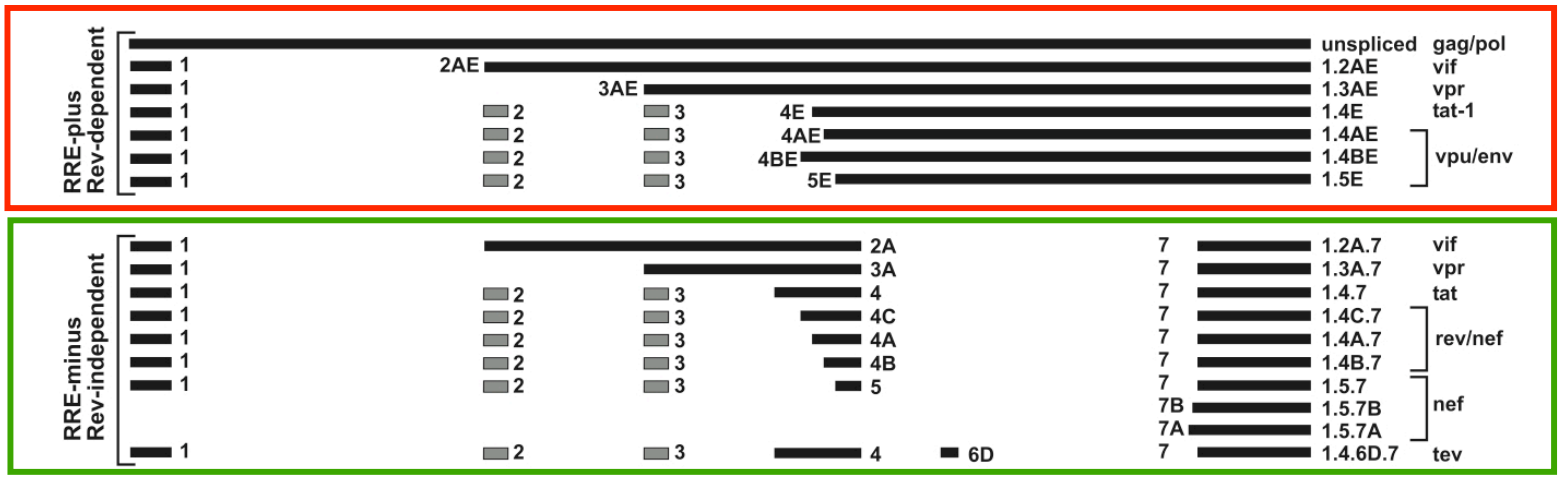

Infection
Virion formation
Export, packaging
RNA
Transcription
DNA
old
RNA
Reverse transcription
DNA
Integration
DNA
new

Organization Of The HIV-1 Genome



HIV-1 mRNAs



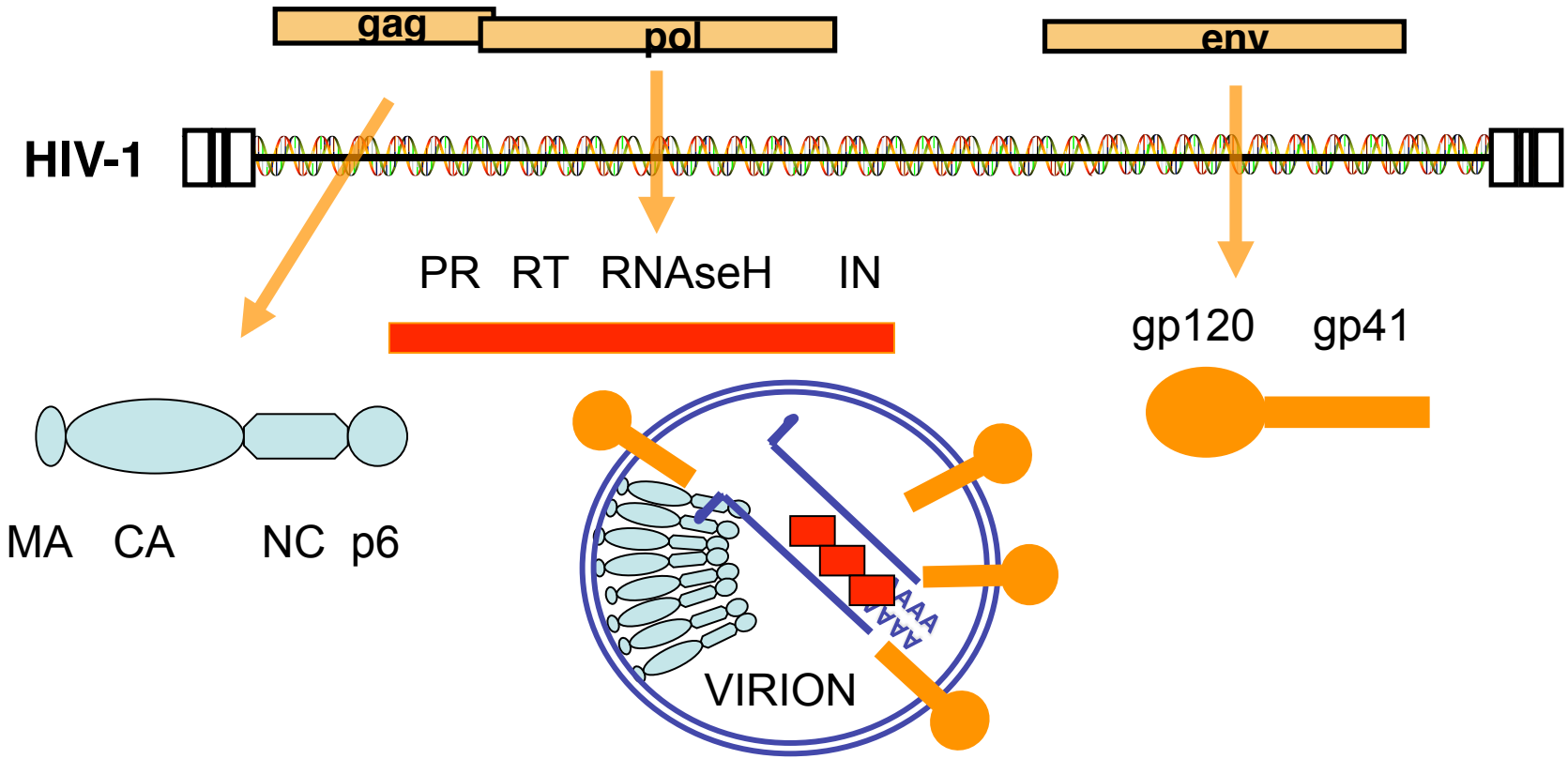


Export mechanism:

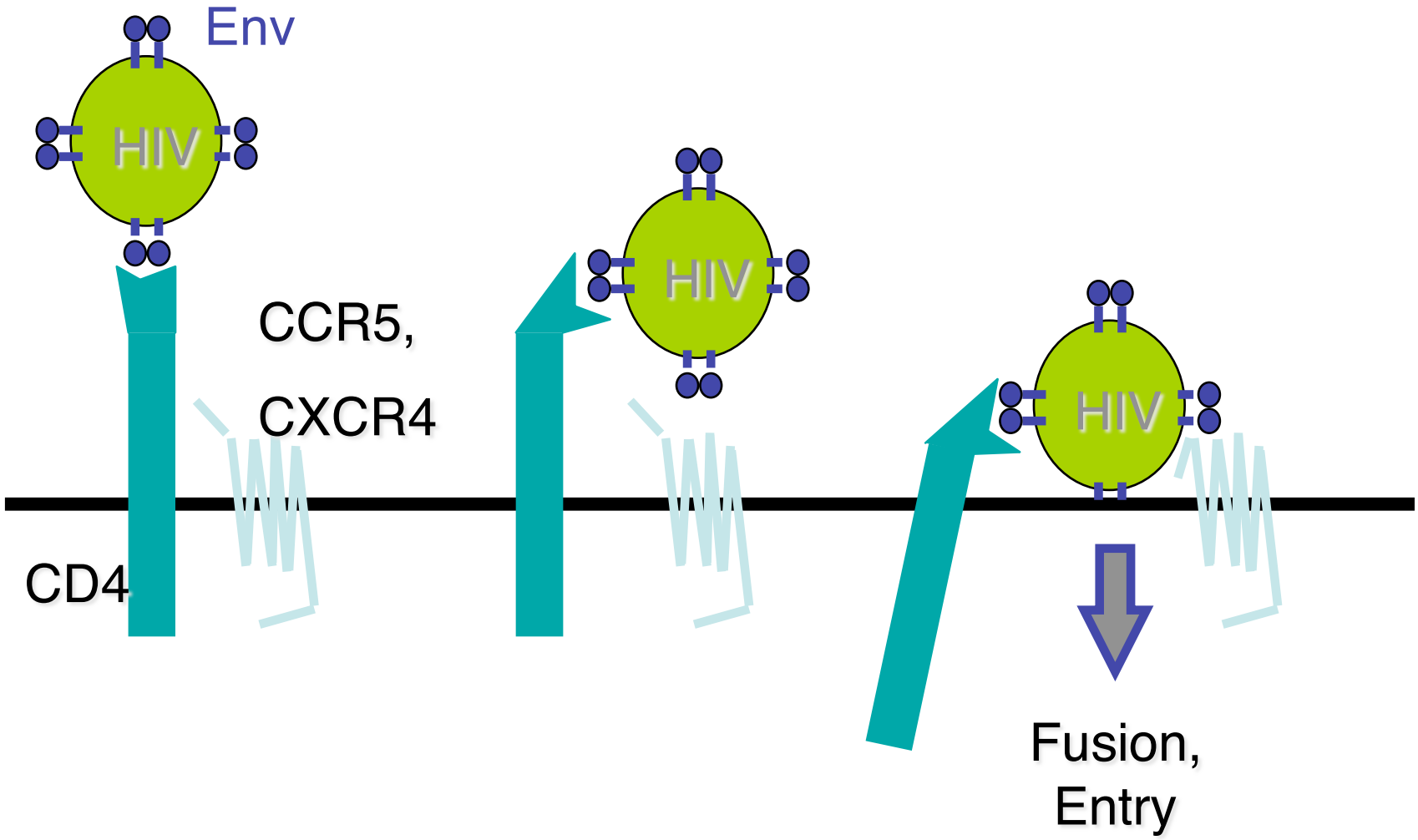
Specialized

Default

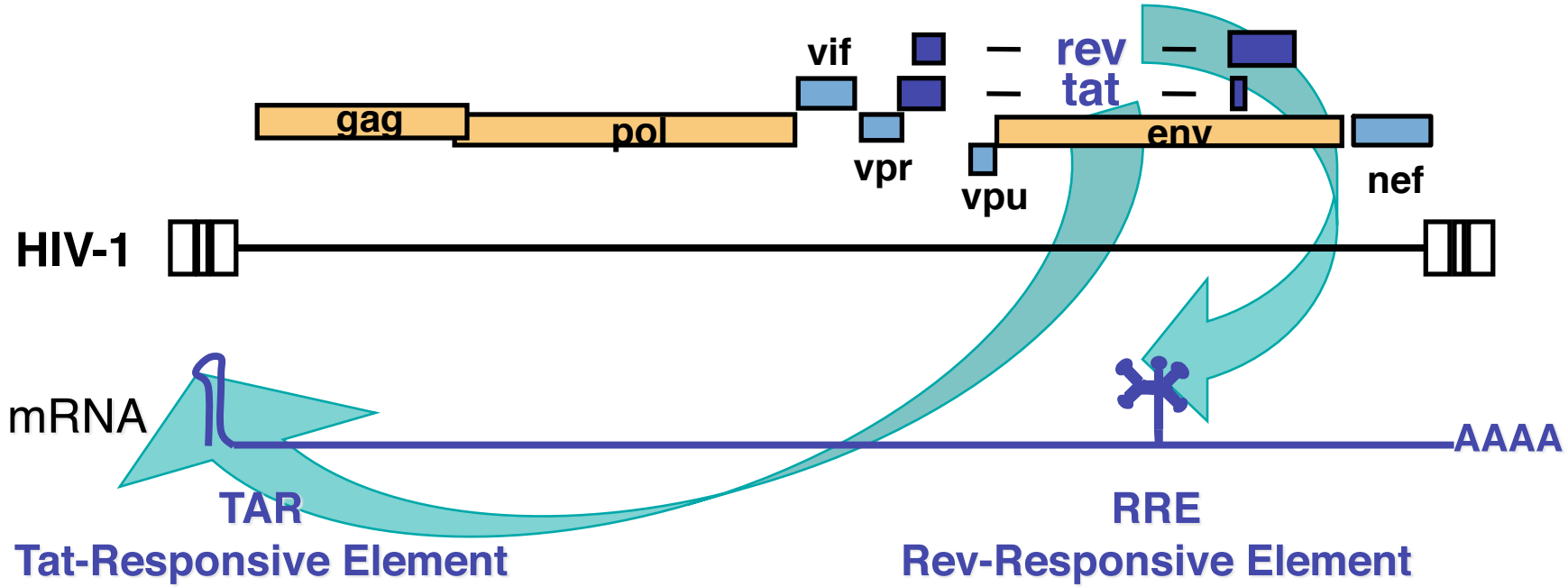
Organization Of The HIV-1 Genome



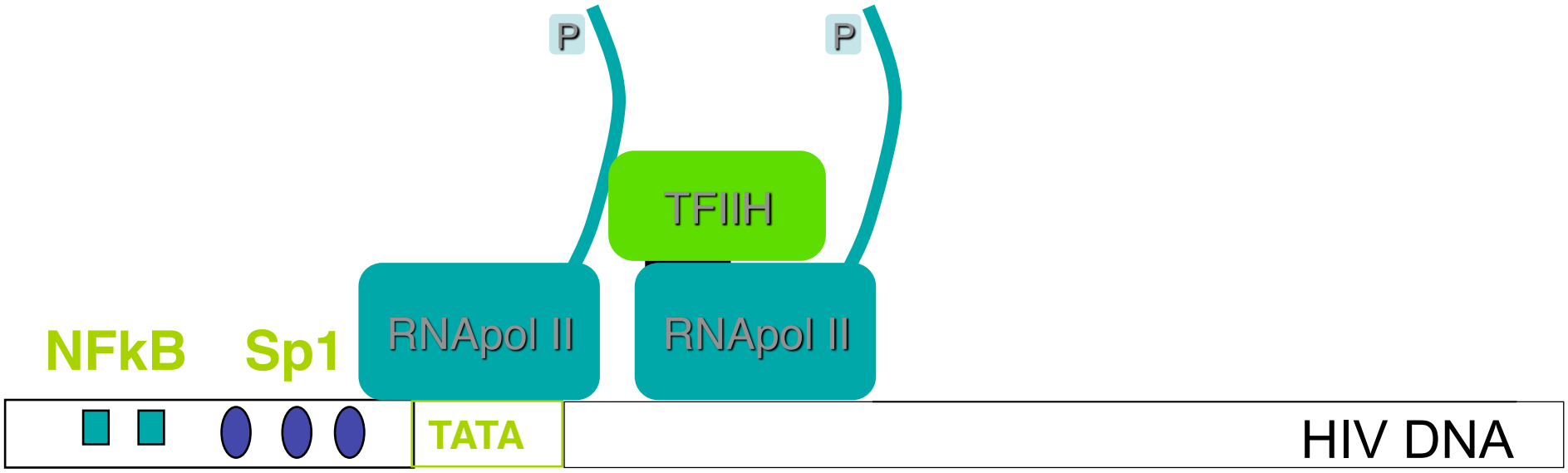
Env And HIV-1 Entry



Tat and Rev Are Essential RNA Binding HIV-1 Proteins

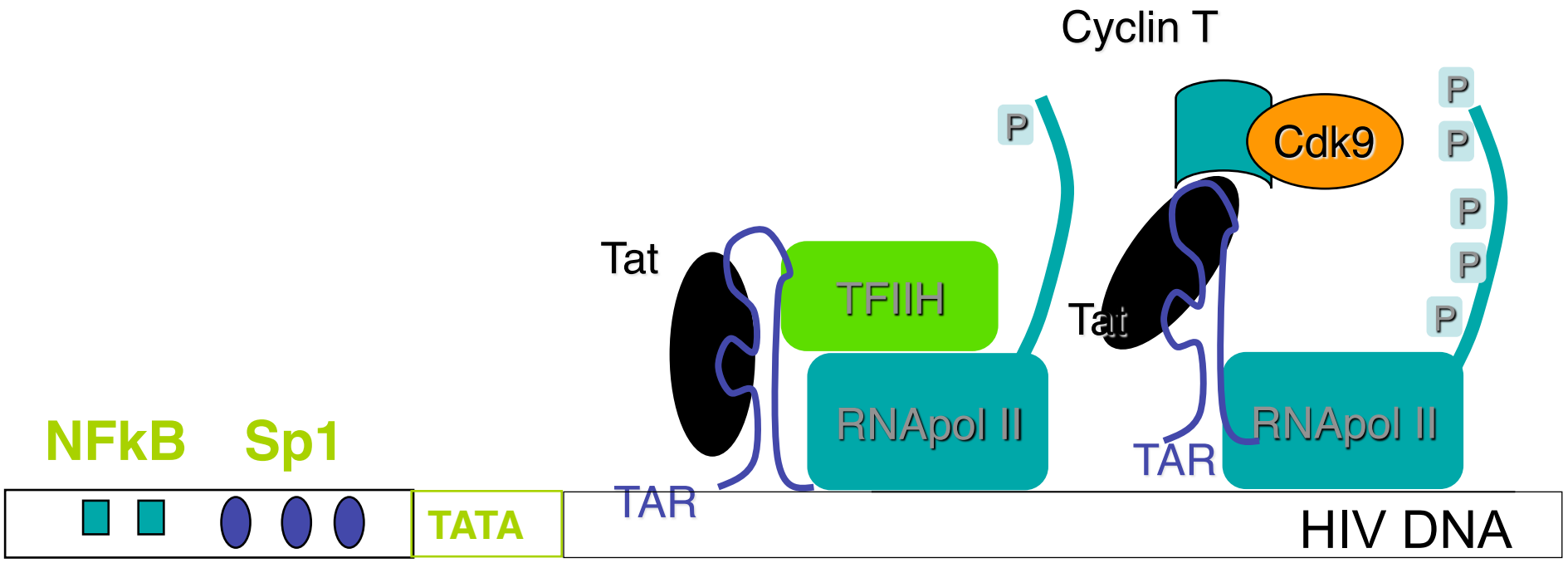


HIV-1 Promoter



Assembly Clearance

HIV-1 Promoter



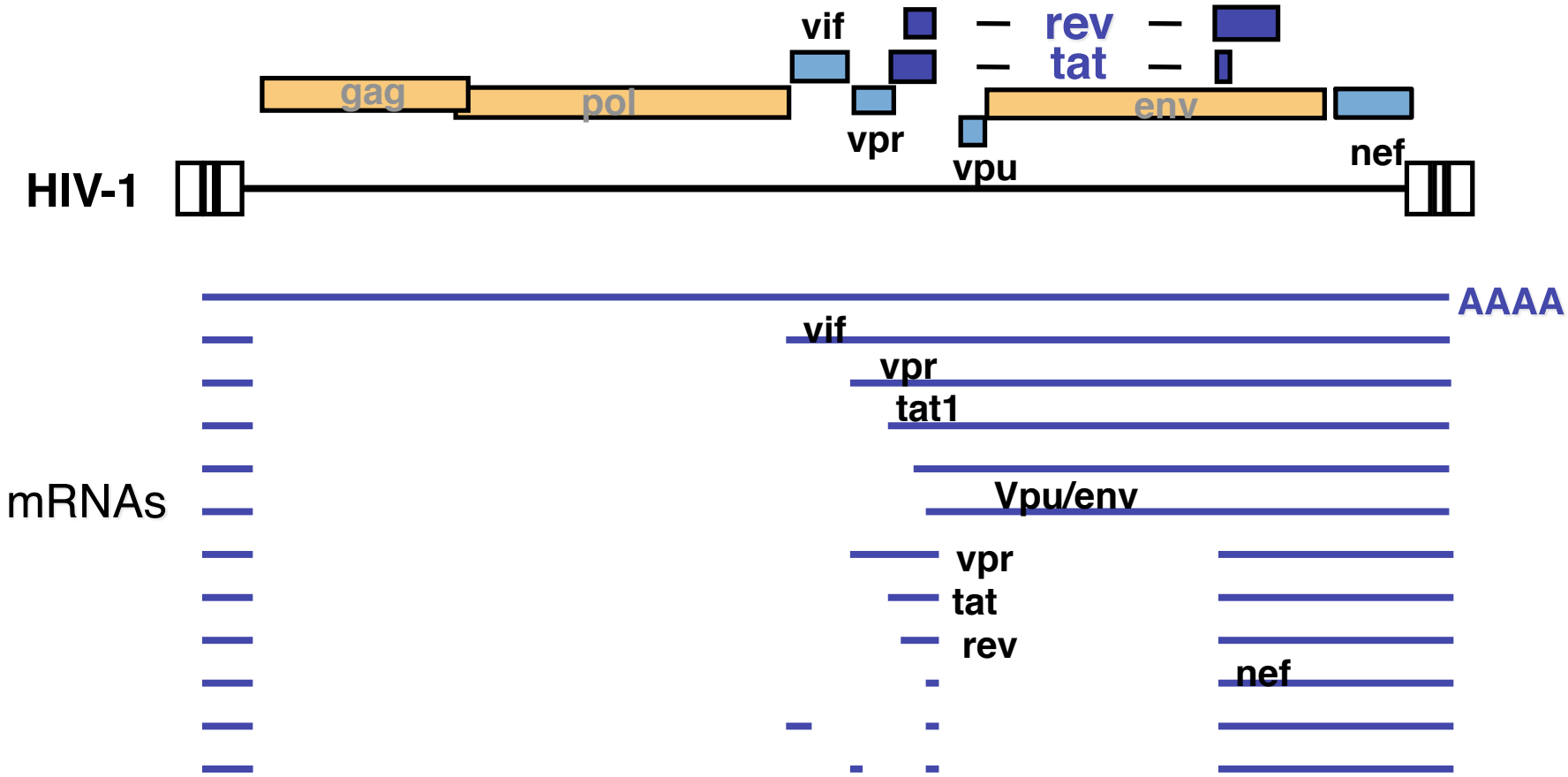
Assembly Clearance Pausing Elongation

HIV-1 mRNA expression

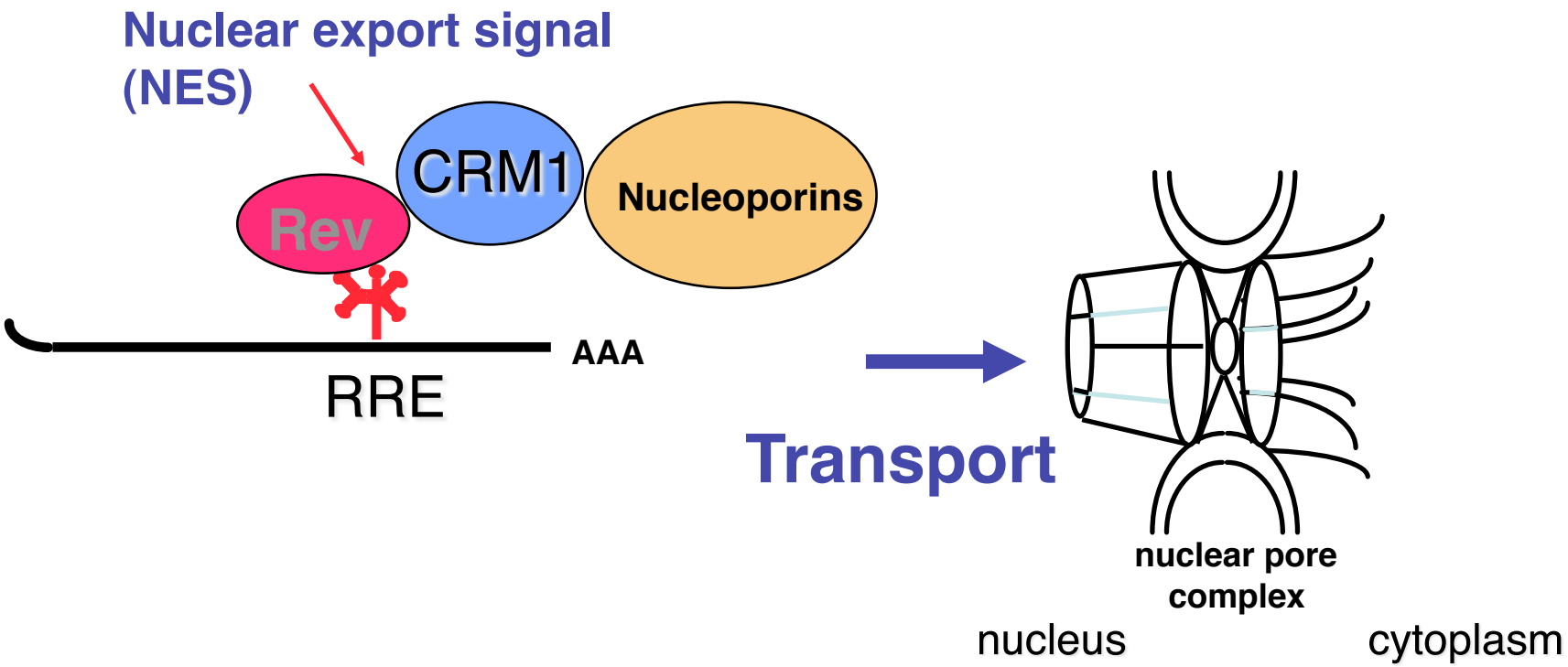
- HIV uses
  - > Alternative splicing
  - > Bicistronic mRNAs
  - > Poly-protein production and processing
  - > frameshifting

to express many proteins from one primary transcript

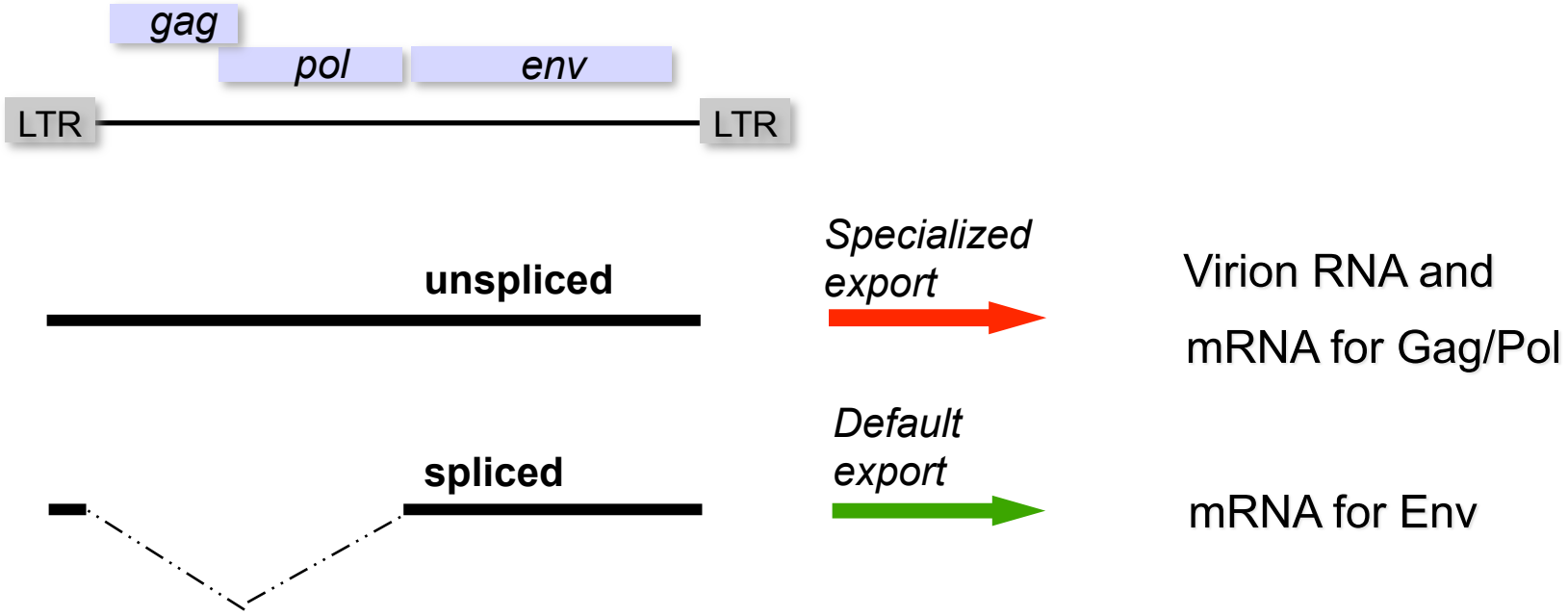
HIV-1 mRNA expression



The Rev/CRM1 mRNA Export Pathway



Retroviruses and Env-containing LTR-retroelements depend on specialized mRNA export mechanisms to express their full-length mRNA



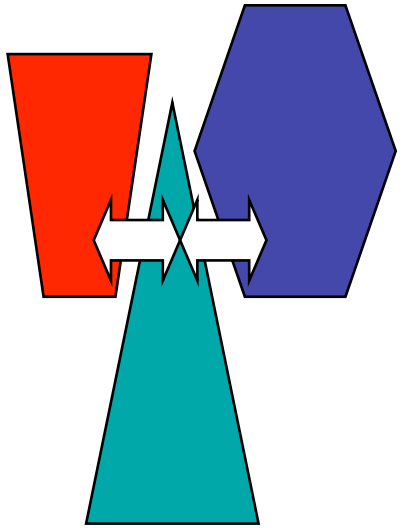
Specialized nuclear export mechanisms are necessary to export the unspliced transcripts that serve as virion RNA and also encode the essential Gag/Pol polyproteins

HIV-1 Regulatory and Accessory Proteins

- Tat — transcriptional transactivator
- Rev — viral mRNA export factor
- Vif — prevents Innate immunity by APOBEC3G
- Vpr — multifunctional, Transactivator, G2 arrest, nuclear import
- Nef — multifunctional, CD4, MHC-1 downregulation
- Vpu — CD4 degradation in ER

HIV-1 Proteins as Molecular Adaptors

	Interactors		
• Tat	Cyclin T	⟺	viral RNA
• Rev	CRM1 (exportin1)		viral RNA
• Nef	CD4, MHC-1		AP1, AP2
• Vif	APOBEC3G		PROTEASOME
• Vpr	TFIIB, hGR, others		p300/CBP
• Vpu	CD4		h-βTrCP



The Importance Of CD4 Regulation

- Several HIV proteins modulate CD4
  - > Env
  - > Nef
  - > Vpu

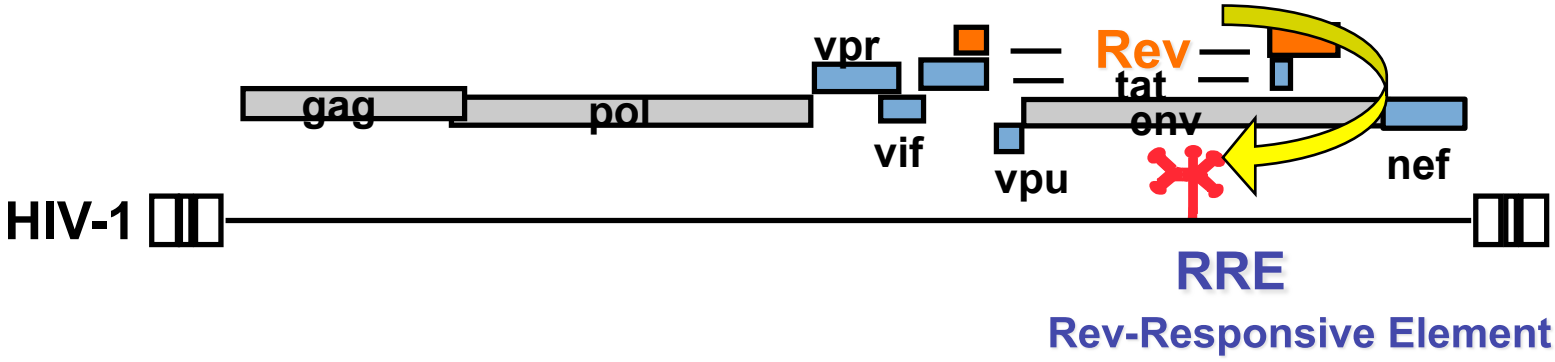
HIV Research Has Benefited Many Fields

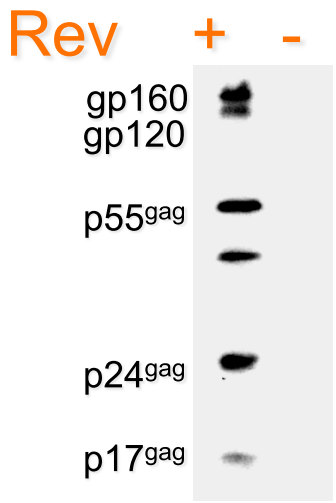
- Control of transcriptional elongation (Tat)
- Macromolecular export from the nucleus (Rev)
- Innate Immunity against retroviruses (Vif)
- Intracellular trafficking of proteins (Gag, Rev, Tat)

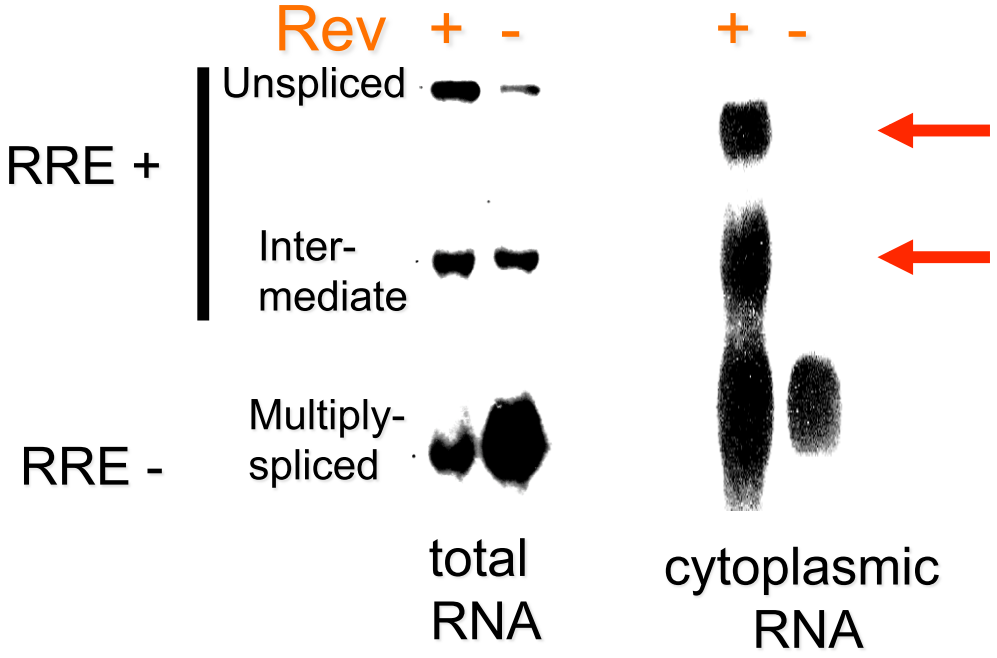
HIV-1 Genome

- Compact
- Highly regulated
- Adapted for high and rapid expression in permissive cells
- Able to integrate, to cause a chronic active infection and to persist indefinitely
- Multiple interactions with cellular factors through viral multifunctional adaptors

Effects of Rev on HIV-1 mRNA and Protein Expression

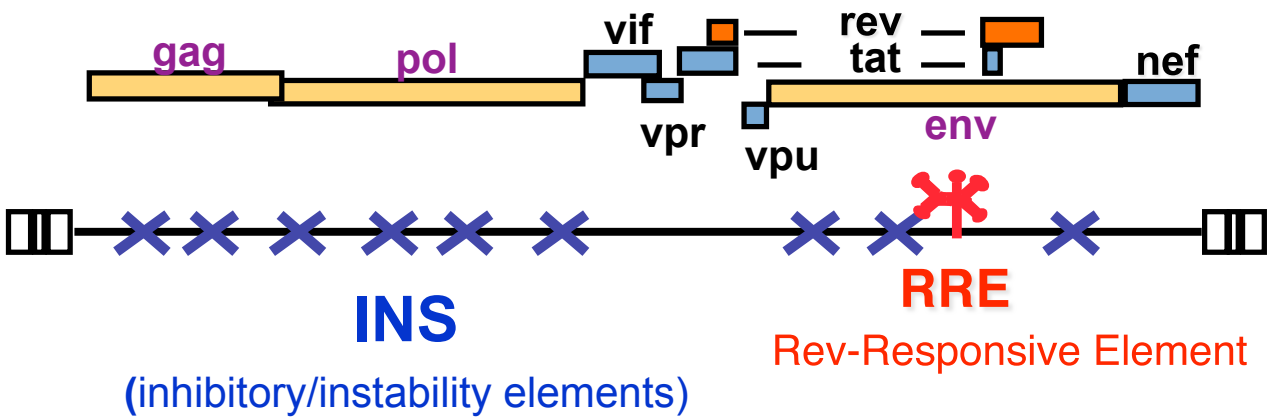






J. Virol. 63: 1265 (1989); *PNAS* 86: 1495 (1989)

Identification of RNA Elements Responsible for Poor Expression of HIV

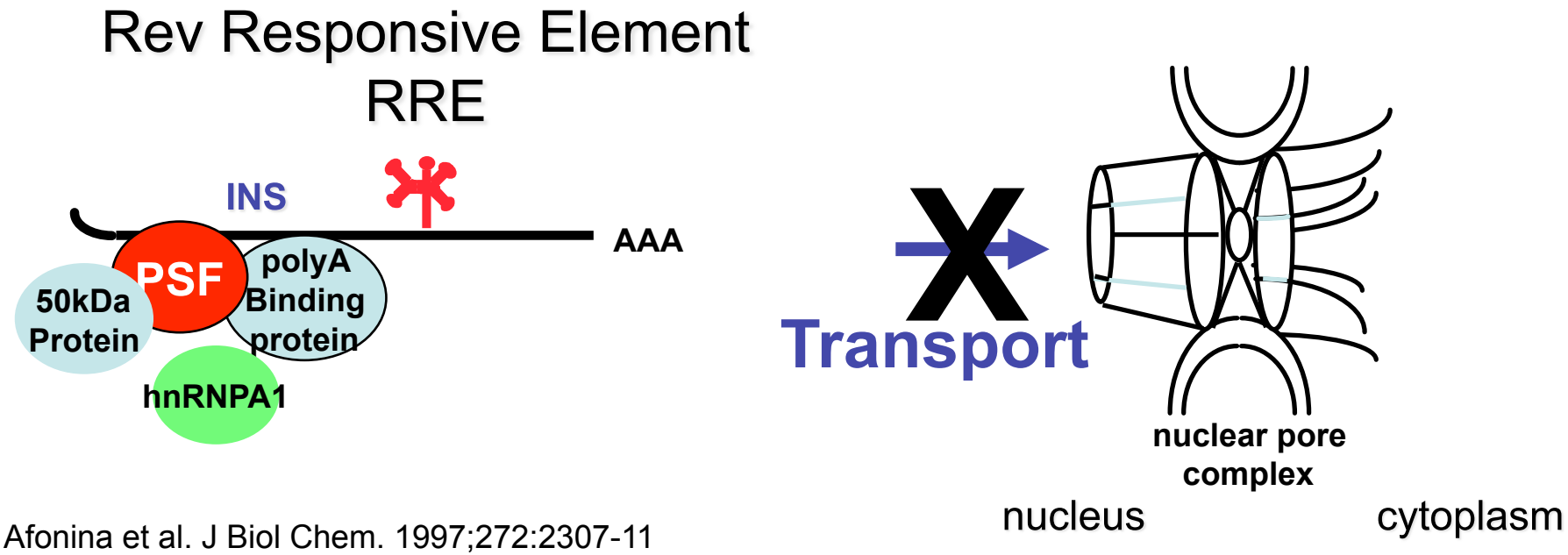


(inhibitory/instability elements)

- INS are negatively acting RNA elements
  - > Are transferable to non-HIV reporter like CAT, GFP
  - > Act when placed outside of open reading frame
- Rev-RRE interaction counteracts the negative-acting INS
- RNA/codon optimization results in highly efficient expression of gag, pol, env
  - > Changing RNA without affecting coding potential
  - > Key methodology to generate expression vectors for HIV vaccine approaches

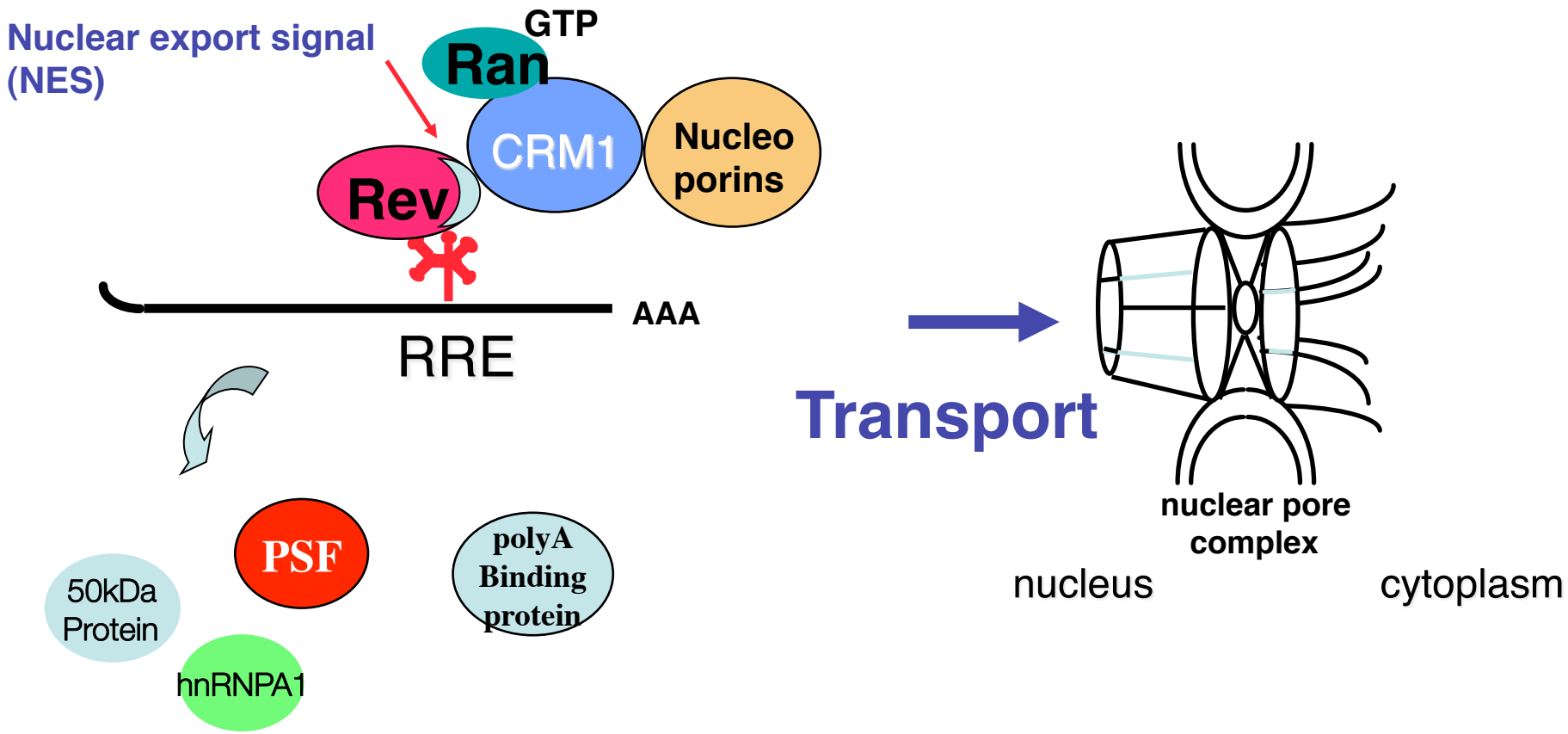
J. Virol. 66: 150 (1992); ibid 66: 7176 (1992;) ibid 68: 2986 1(994); ibid 71: 4892 (1997)

Inhibitory Sequences Interact With Specific Factors In The Nucleus

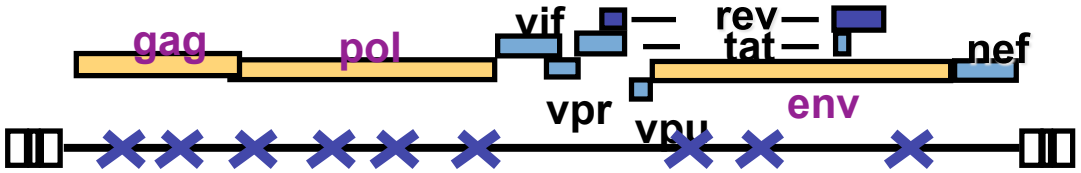


Afonina et al. J Biol Chem. 1997;272:2307-11
Najera et al J Mol Biol. 1999;285:1951-64
Zolotukhin et al Mol Cell Biol. 2003;23:6618-30

The Rev/CRM1 mRNA Export Pathway



Generation of Efficient Vectors for HIV/SIV Protein Expression by RNA/Codon Optimization



INS (inhibitory/instability elements)

Two ways to eliminate inhibitory/instability elements (INS):

- Bypass the effect of INS by co-expression of Rev protein
- Provide alternative post-transcriptional control elements CTE, RTE

- Elimination of INS is achieved by introducing multiple point mutations in the coding regions (RNA optimization/codon optimization)

  > RNA optimization leads to dramatically increased protein expression and is an essential component of DNA vaccines

  [Schwartz, et al. (**1992**) J Virol. **66**: 150-159; J Virol. **66**: 7176-7182]

  (first generation of DNA vaccine vectors)

Posttrancriptional (RNA) Optimization: Stable mRNA=Better Protein Expression

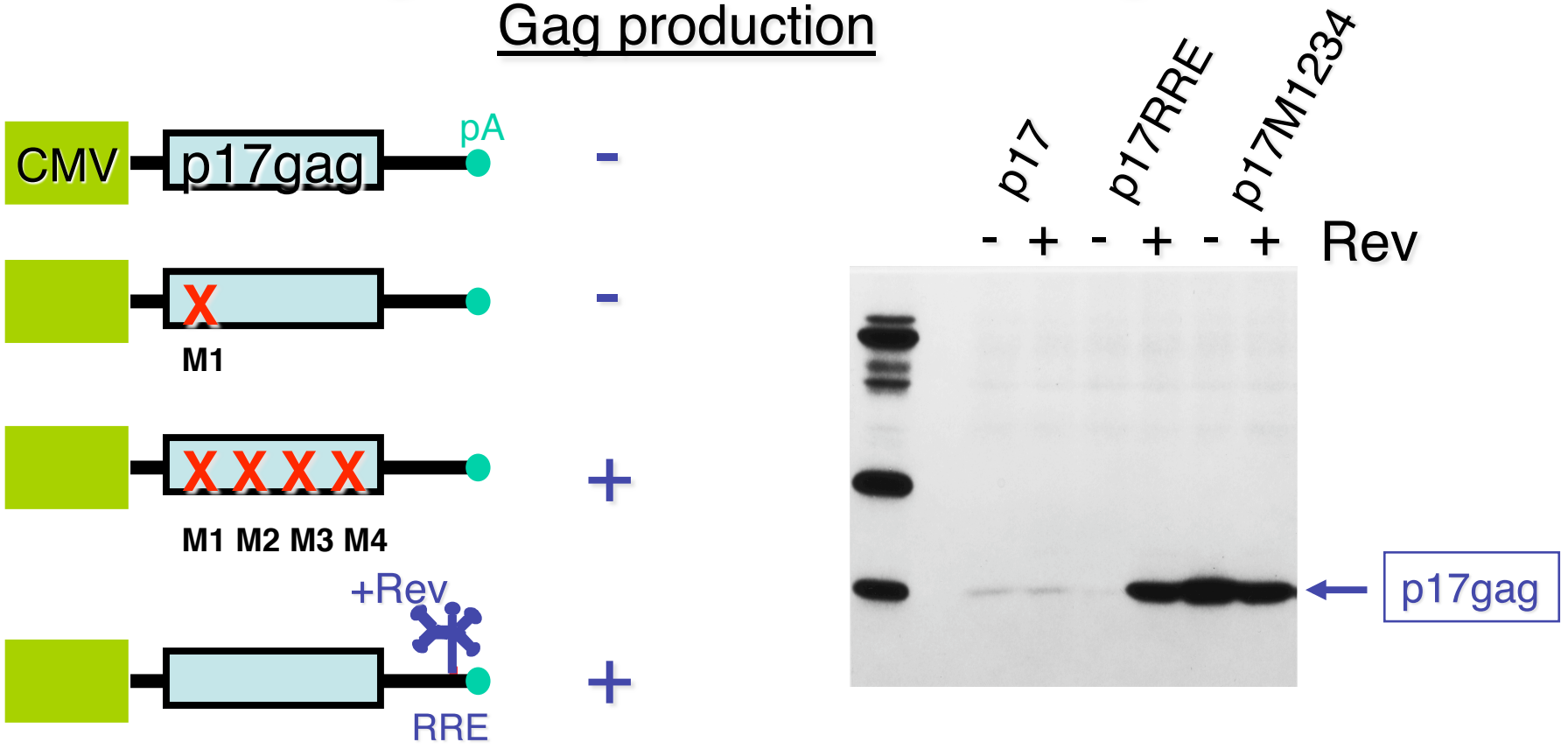
…AAA AAA TAT AAA TTA AAA CAT ATA….. WT

…AAG AAG TAC AAG CTA AAG CAC ATC….. optimized

Lys Lys Tyr Lys Leu Lys His Ile

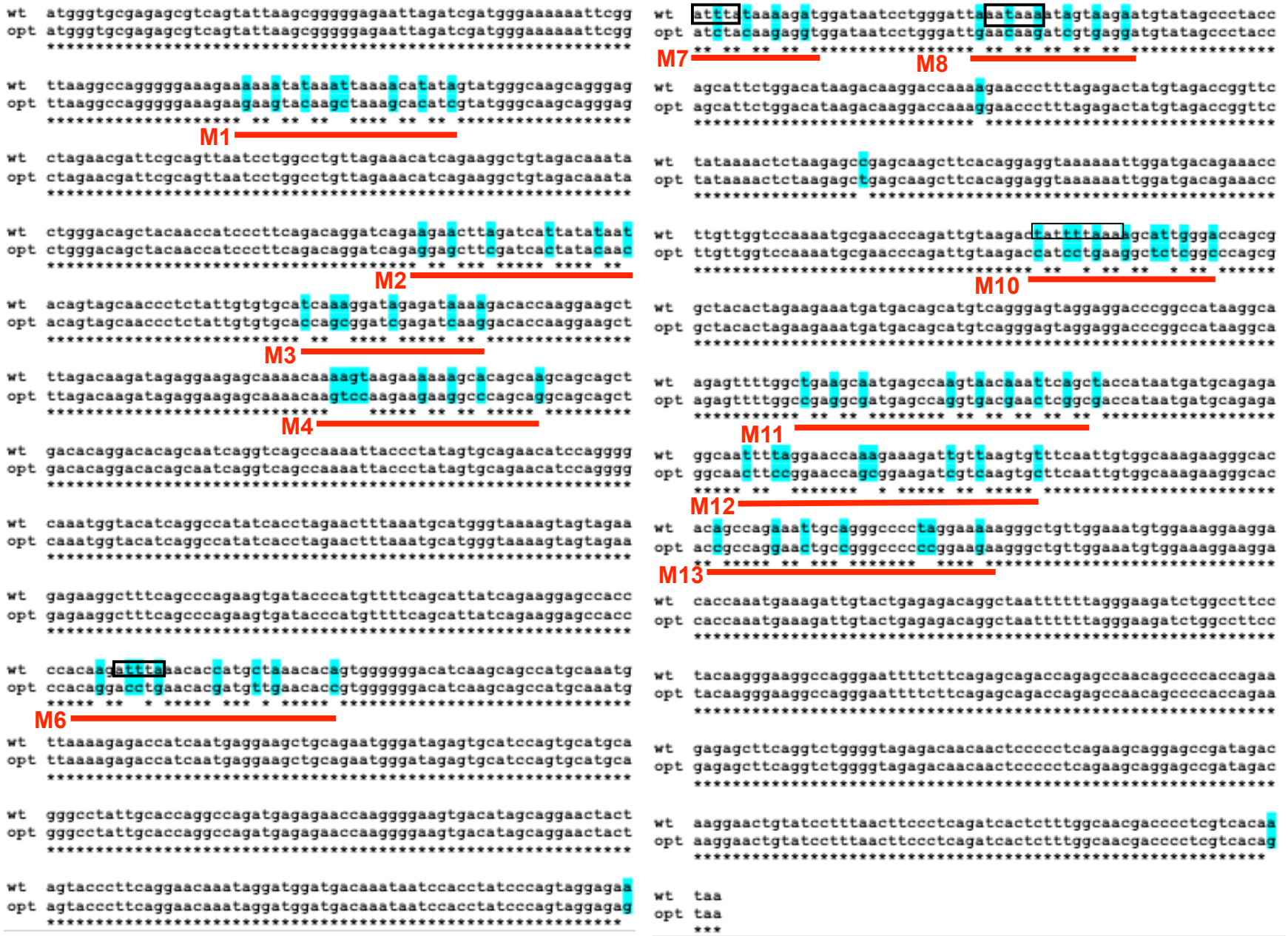
Changes in multiple codons result in stable mRNA, efficiently exported and translated in the ribosome

Inhibitory Sequences In Coding Regions of HIV-1 Gag

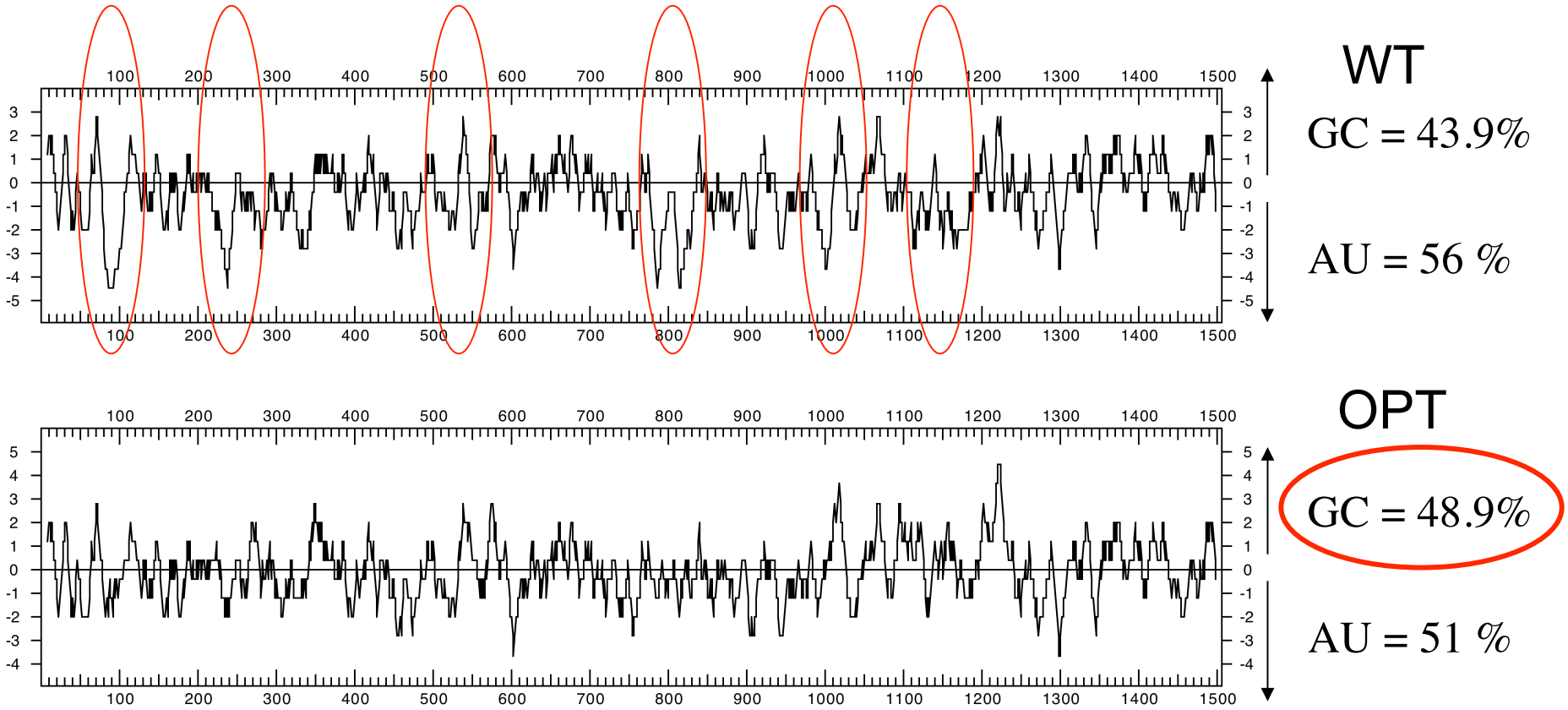


Optimization of HIV-1 gag

94.4% nt identity
(84/1503 nt changed)

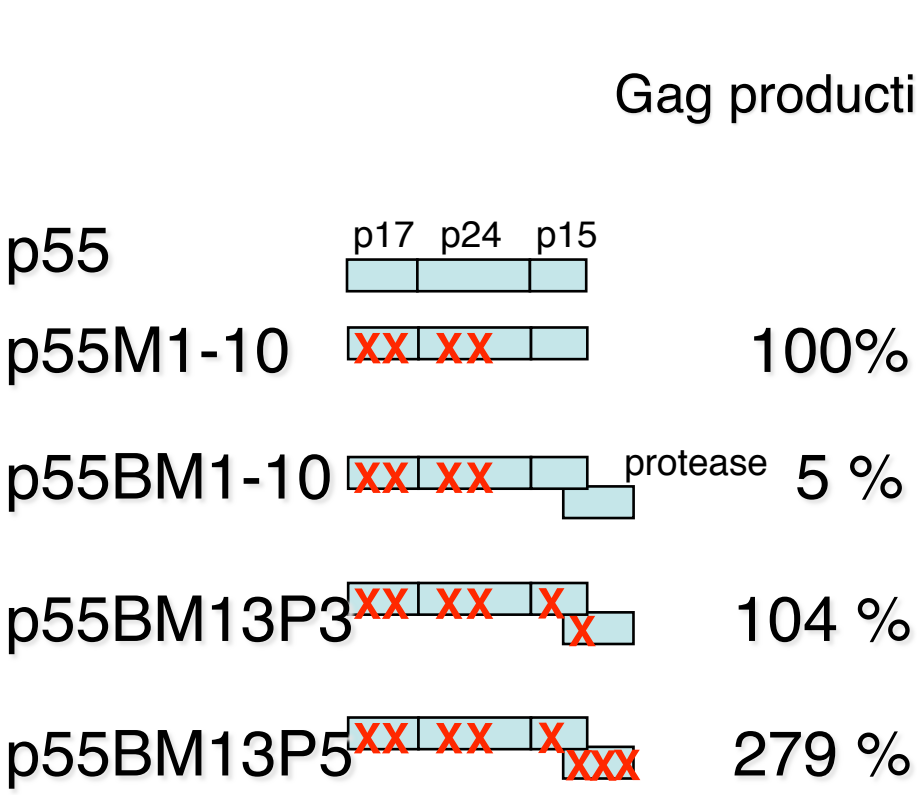


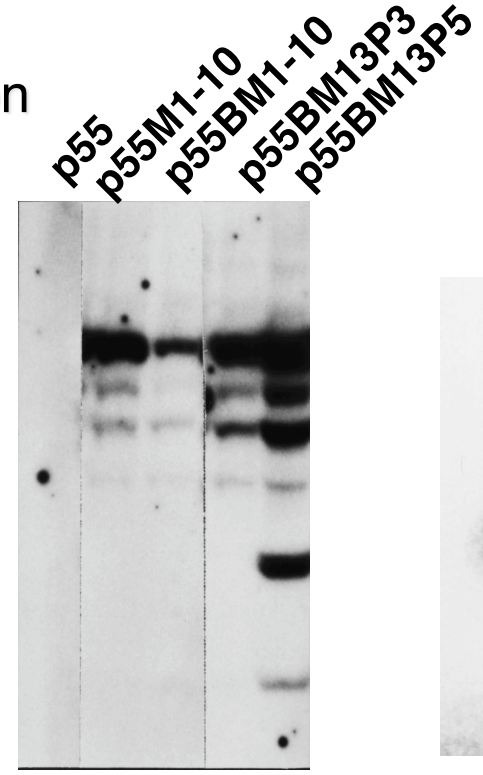
AU- and GC-profile of HIV gag

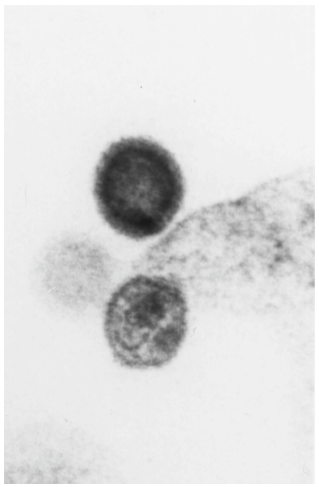


Optimized gag mRNA has increased GC-content

High Level of Gag Protein and Particle Production By INS(-) Gag Expression Vectors





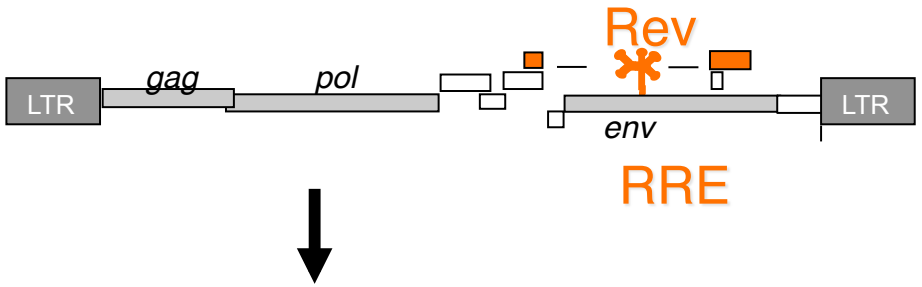


Production of Immature and Mature particles

(Schneider R. et al J Virol (1997): 71, 4892-903)

Posttranscriptional Regulation Is Essential for HIV Expression

HIV or SIV



RNA export via:

Viral Rev protein

1. Elimination of negative acting sequences (RNA optimization) in gag/pol and env results in Rev-independent efficient expression plasmids

- Development of optimized DNA vectors for in vivo DNA delivery

2. Replacement of the Rev regulation by posttranscriptional regulatory system of SRV-1 results in live-attenuated non-pathogenic virus

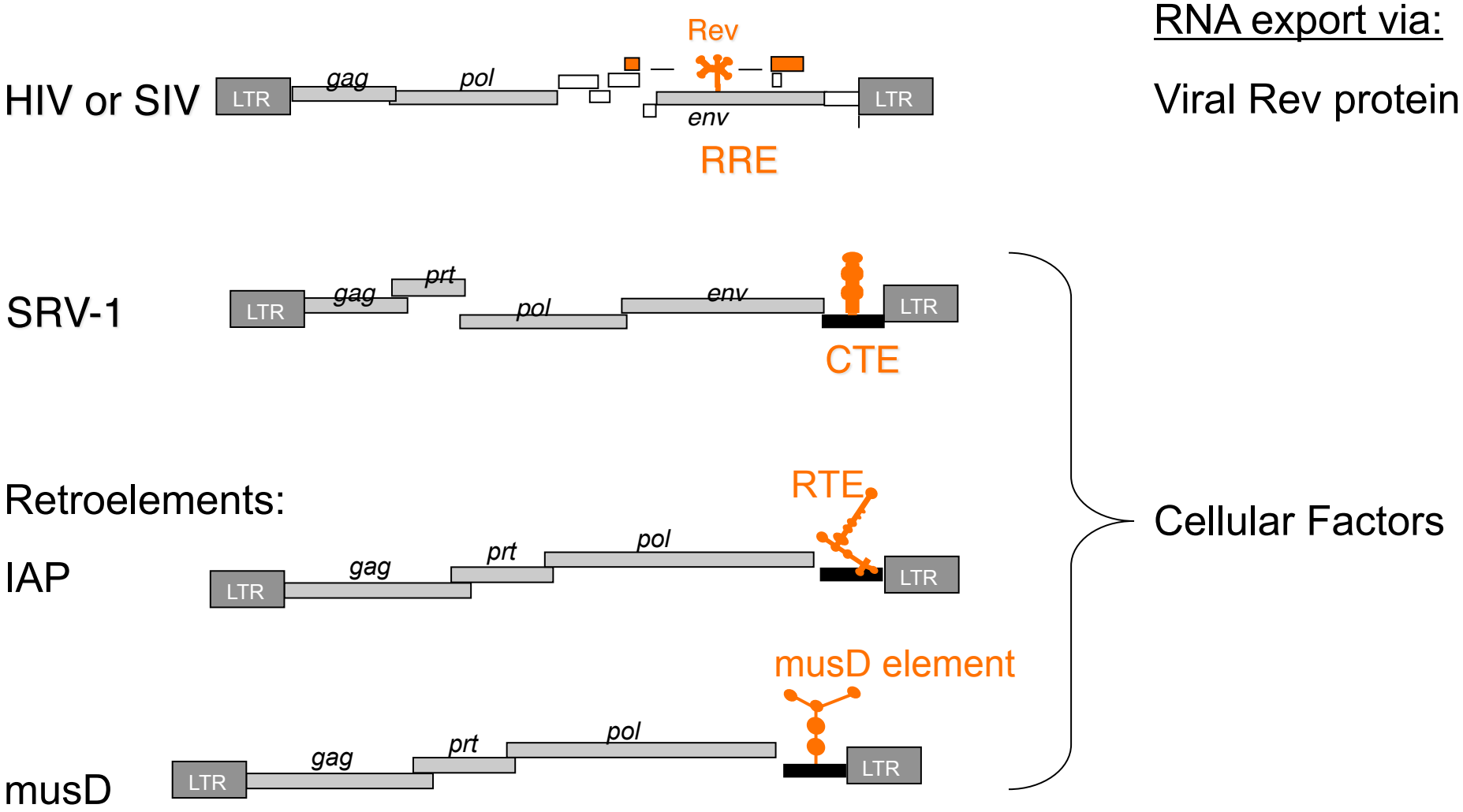
- model to study mechanisms mediating protective immunity against SIV

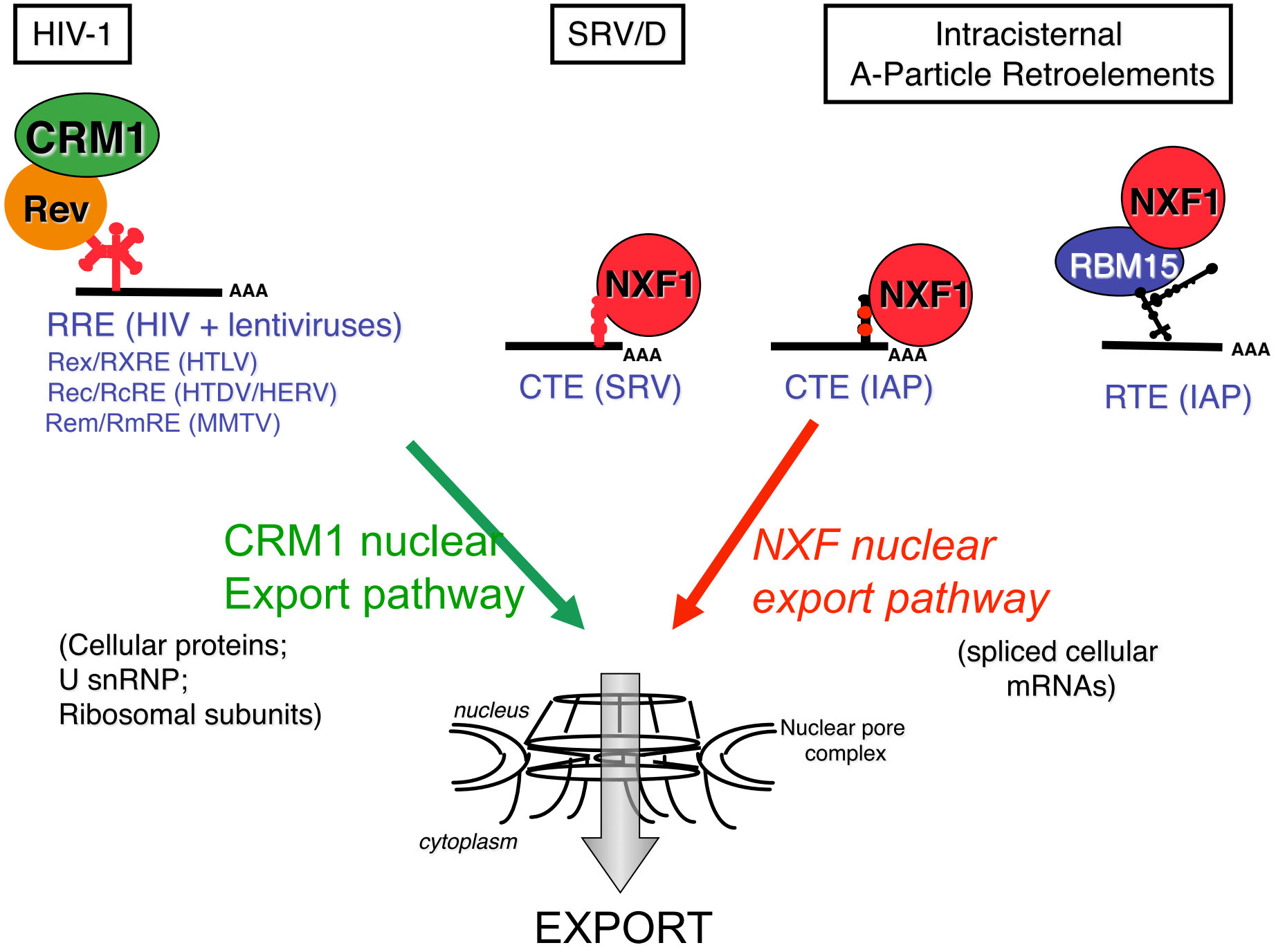
“We Keep Learning from Retroviruses”

Retroviruses provide important clues to understand distinct transport pathways used to export cellular proteins and mRNAs

- Discovery of novel RNA export elements and RNA export factors
- Discovery of mechanisms of function of cellular factors involved in retroviral posttranscriptional regulation

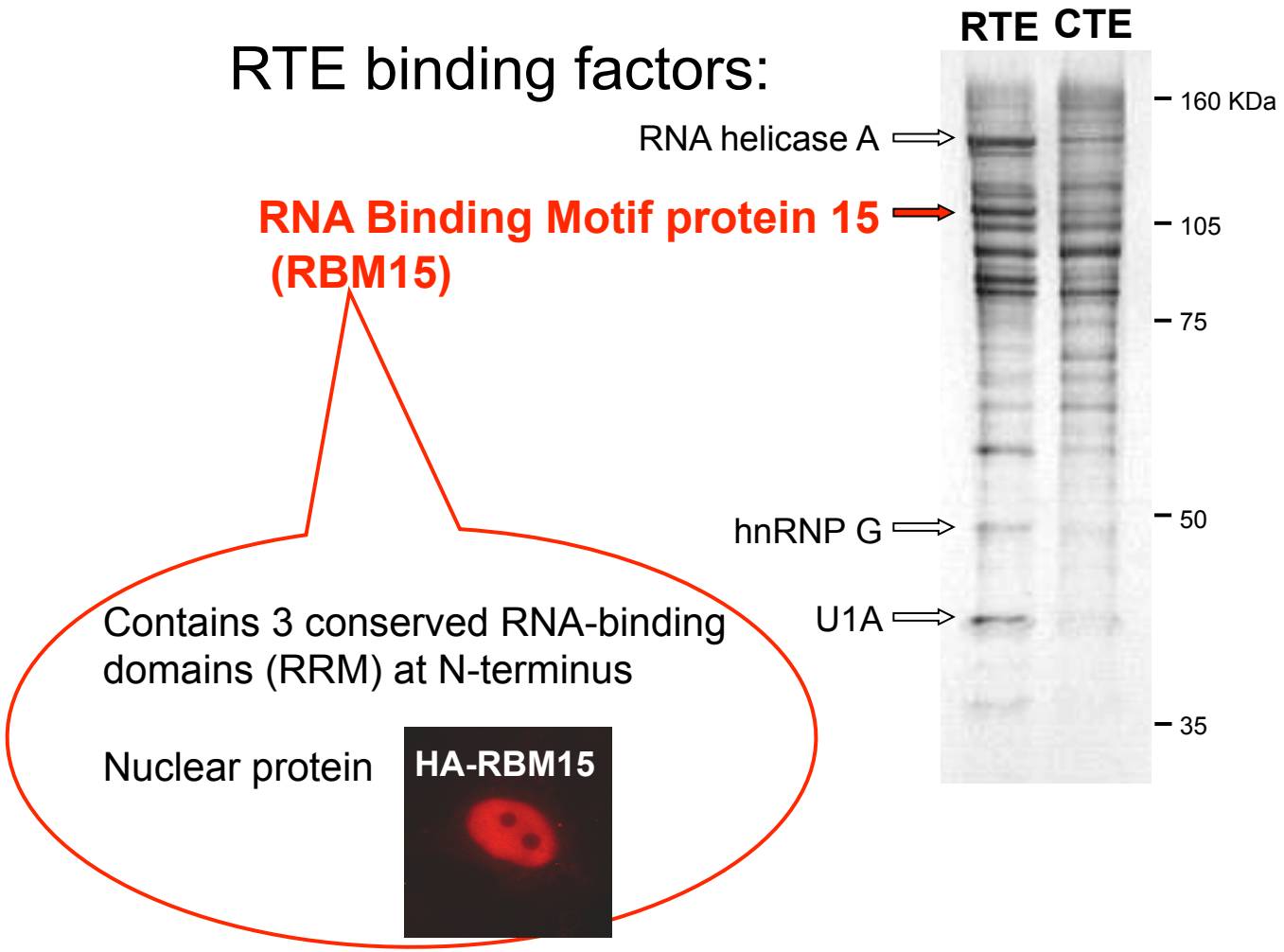
Posttranscriptional Regulation Is Essential for Retrovirus and Retroelement Expression




HIV-1
SRV/D
Intracisternal
A-Particle Retroelements
CRM1
Rev
AAA
RRE (HIV + lentiviruses)
Rex/RXRE (HTLV)
Rec/RcRE (HTDV/HERV)
Rem/RmRE (MMTV)
NXF1
AAA
CTE (SRV)
NXF1
AAA
CTE (IAP)
NXF1
RBM15
AAA
RTE (IAP)
CRM1 nuclear
Export pathway
NXF nuclear
export pathway
(Cellular proteins;
U snRNP;
Ribosomal subunits)
(spliced cellular
mRNAs)
nucleus
Nuclear pore
complex
cytoplasm
EXPORT

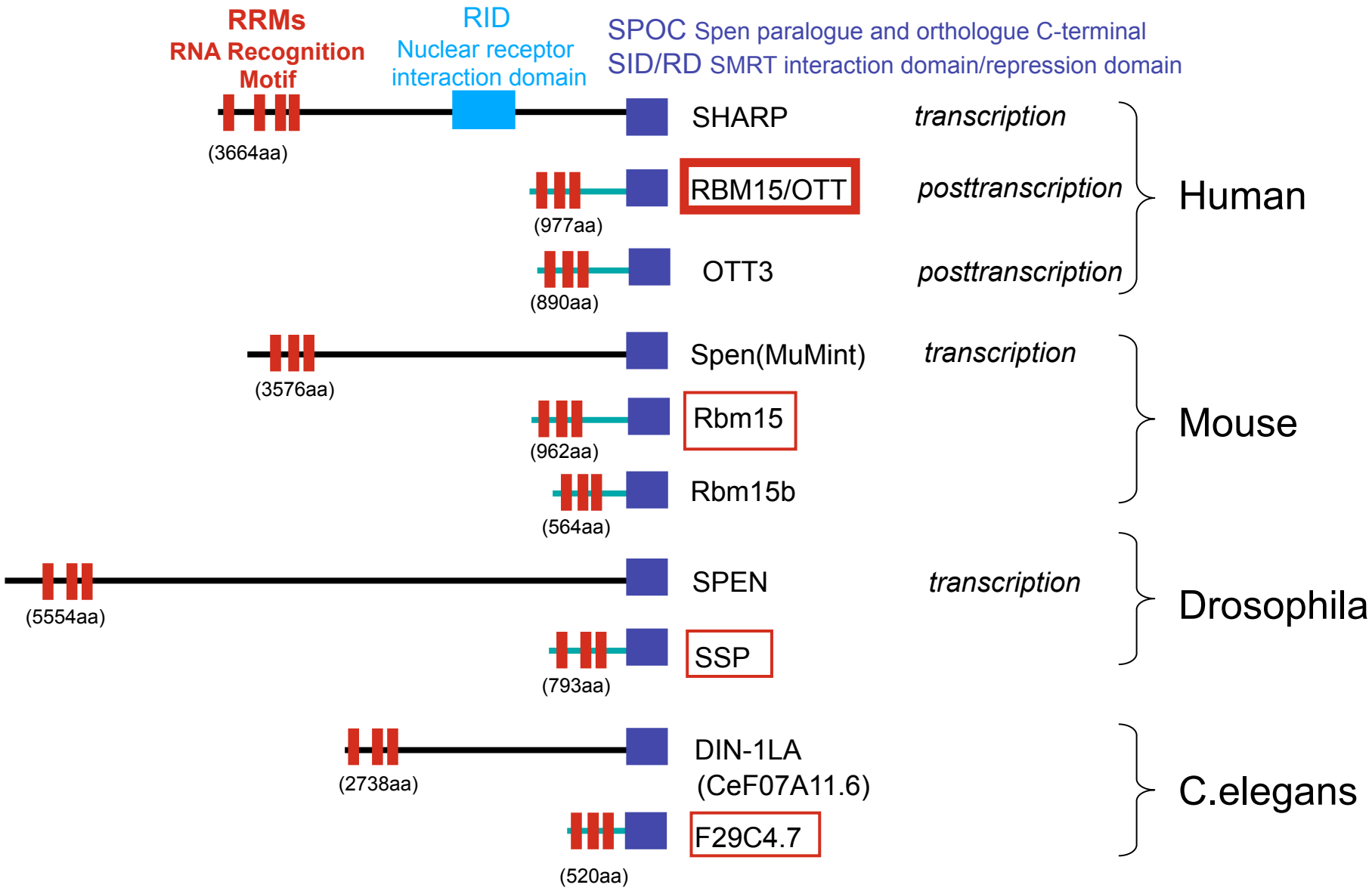
Identification of the Cellular Factors Binding to RTE

RTE binding factors:

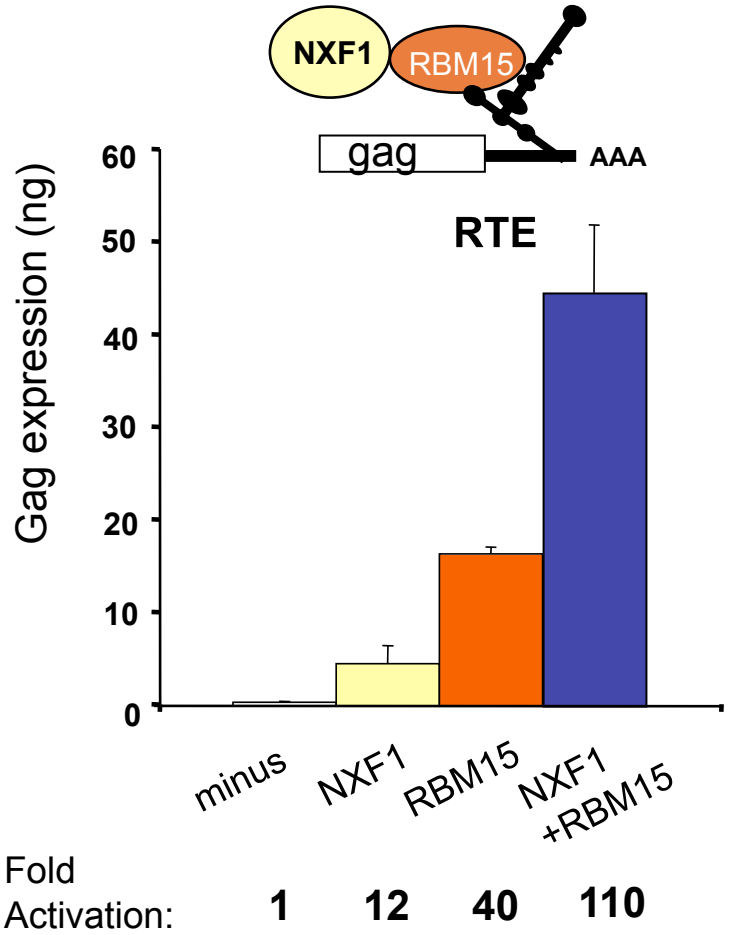


Lindtner et al J Biol Chem 281:36915-28, 2006

RBM15 belongs to Spen (Split end) Family of Proteins

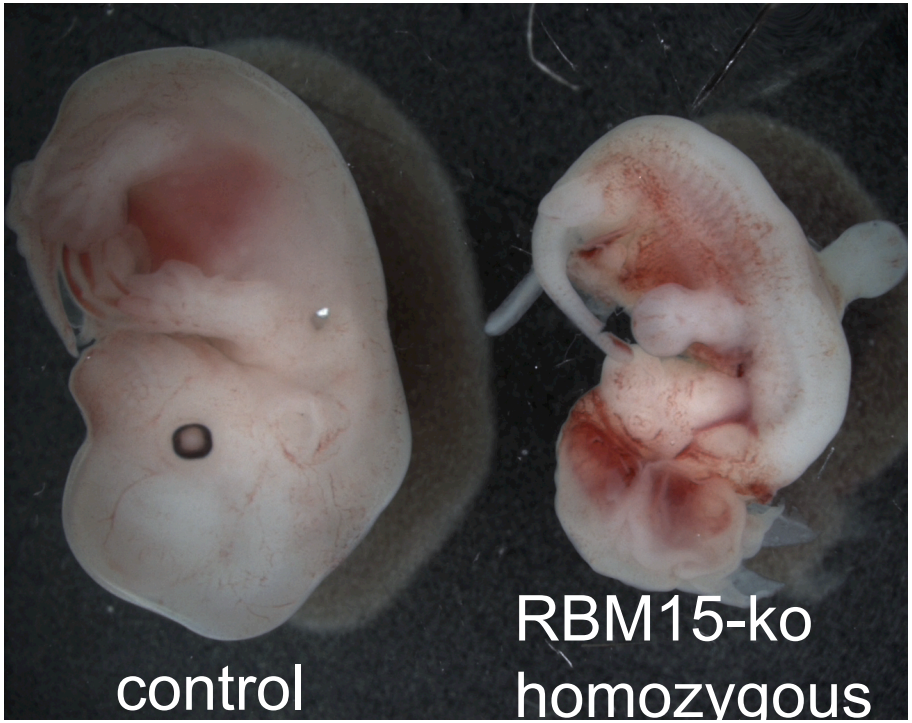


RBM15 Tethers RTE-RNA to the NXF1 Export Pathway



RBM15 and NXF1 interact *in vitro* and *in vivo*, and act cooperatively

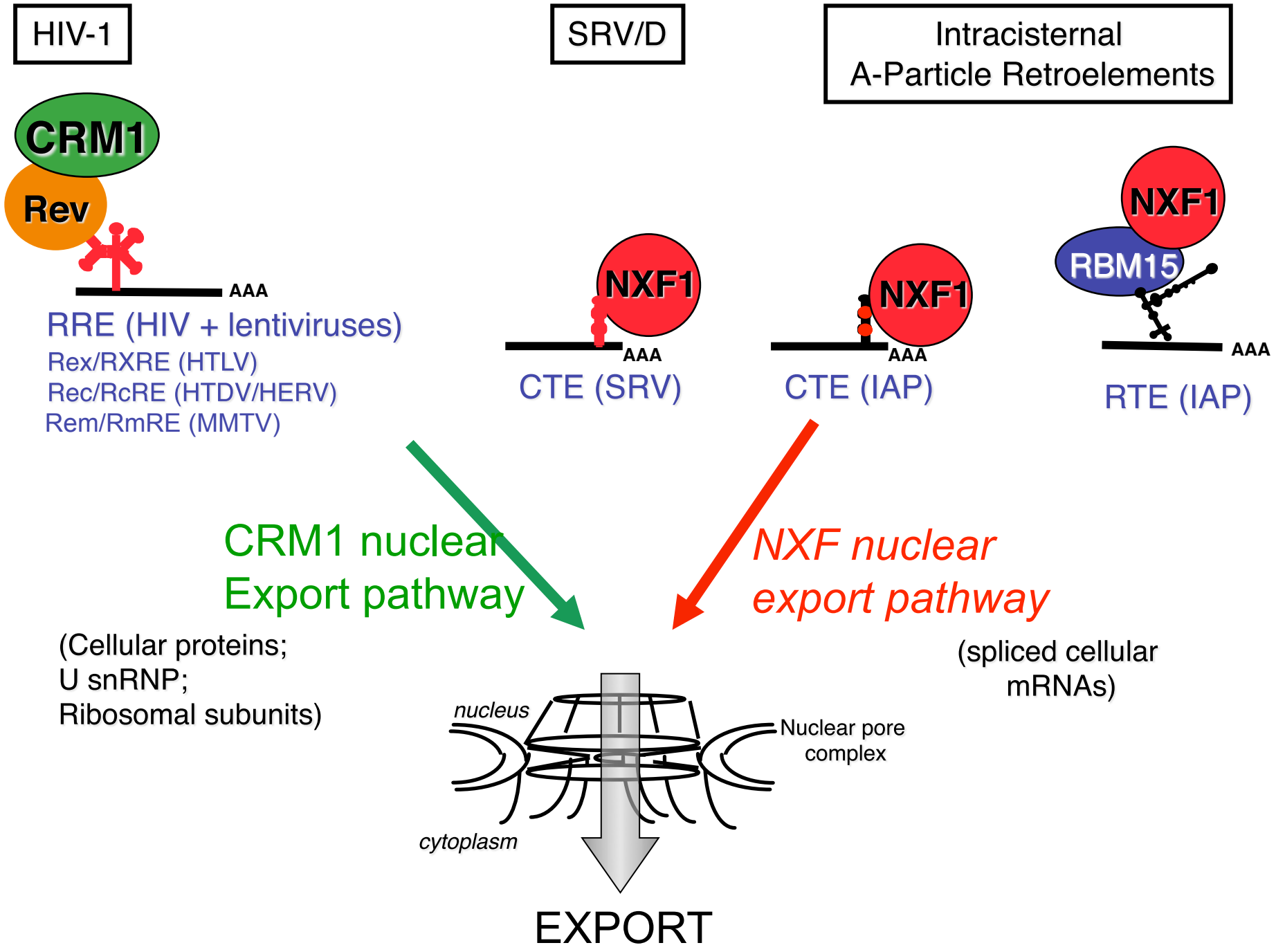
RBM15 is Essential for Mouse Development



Day 11.5

Preliminary study indicates a role of RMB15 in the expression of a subset of cellular mRNAs

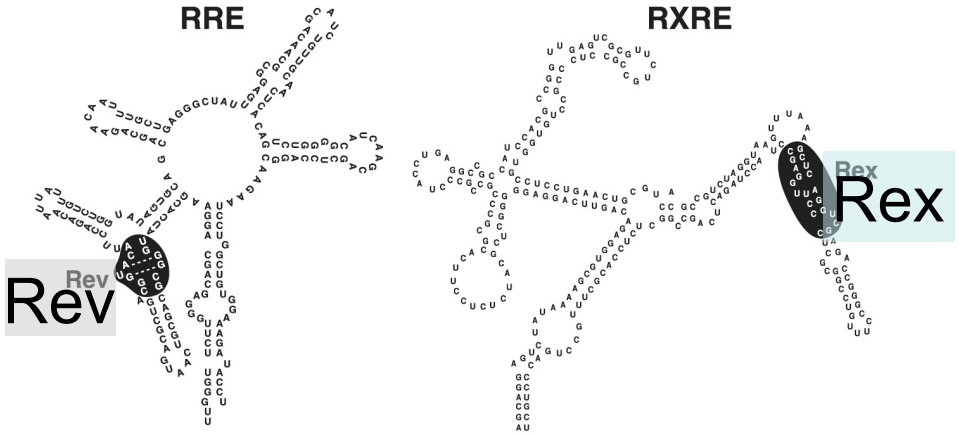
Soren Warming, Nancy Jenkins, Neal Copeland
Mouse Cancer Genetics Program


HIV-1
SRV/D
Intracisternal
A-Particle Retroelements
CRM1
Rev
AAA
RRE (HIV + lentiviruses)
Rex/RXRE (HTLV)
Rec/RcRE (HTDV/HERV)
Rem/RmRE (MMTV)
NXF1
AAA
CTE (SRV)
NXF1
AAA
CTE (IAP)
NXF1
RBM15
AAA
RTE (IAP)
CRM1 nuclear
Export pathway
NXF nuclear
export pathway
(Cellular proteins;
U snRNP;
Ribosomal subunits)
(spliced cellular
mRNAs)
nucleus
Nuclear pore
complex
cytoplasm
EXPORT

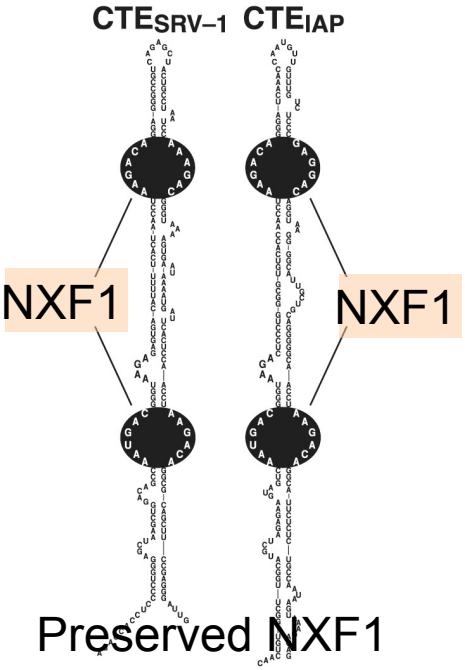
Retroviruses and Retroelements Utilize Distinct RNA Export Mechanisms

Virus	RNA Export element	Viral Export Factor	Cellular Export Factor	Nuclear receptor	
HIV-1	RRE	Rev	N/A	CRM1	*
HTLV-I	RXRE	Rex	N/A	CRM1	
HTDV/HERK	RcRE	Rec	N/A	CRM1	
MMTV	RmRe	Rem	N/A	CRM1	
SRV/D	CTE	no	NXF1	NXF1	*
IAP	CTE_{IAP}	no	NXF1	NXF1	*
IAP	RTE	no	RBM15	NXF1	*
MusD	MusD element	no	RBM15	NXF1	*
RSV	DR	no	?	?	
MLV	?	no	?	?	

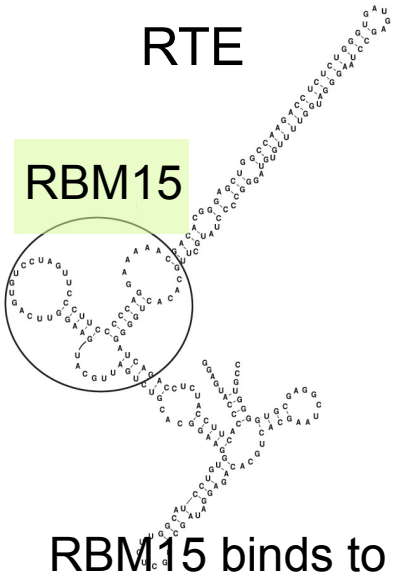
Diversity of Retroviral RNA Export Elements

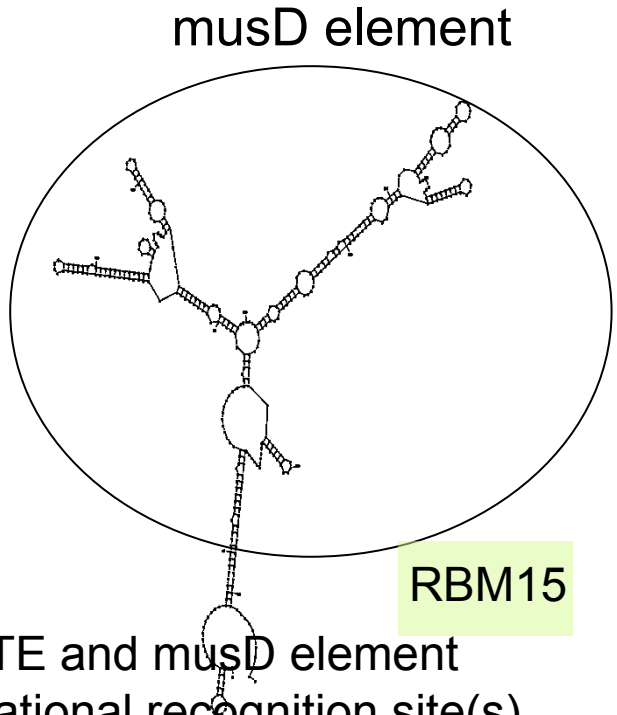


- Different sequence
- Different RNA structure
- Different binding factors



Preserved NXF1 recognition sites

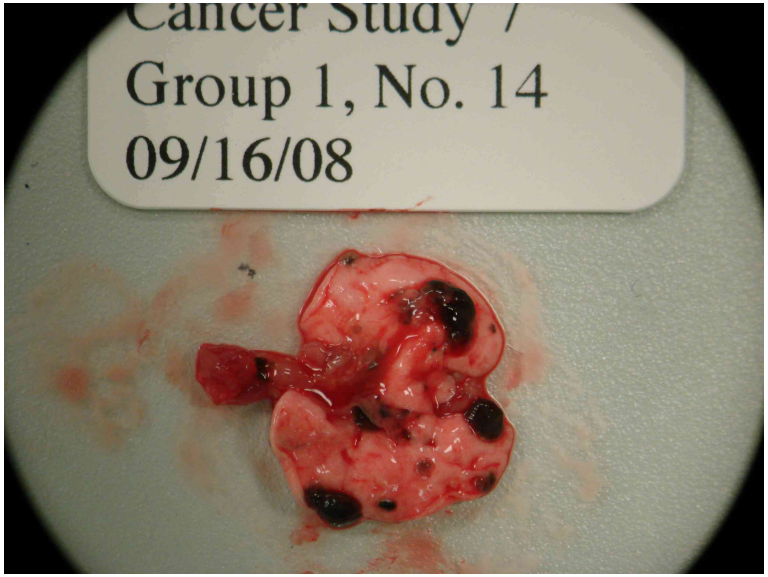




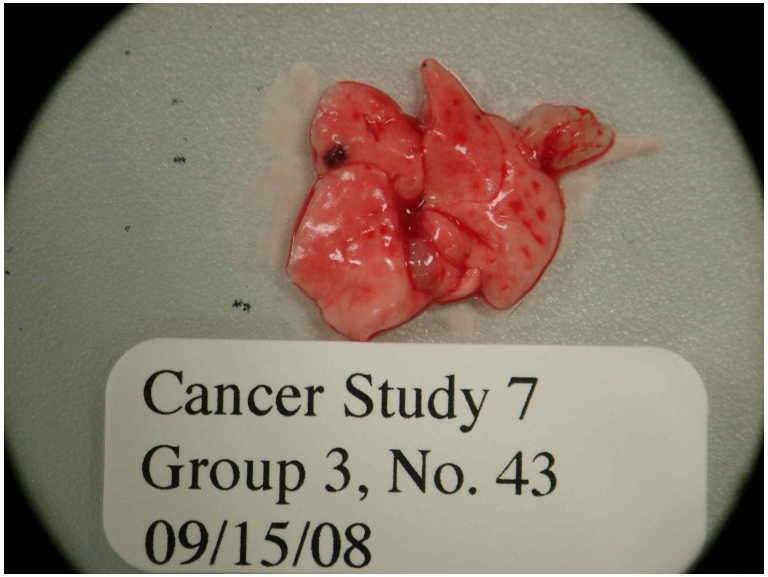
RBM15 binds to RTE and musD element
- suggests conformational recognition site(s)

Study Cancer 7: **LUNG TUMOR MODEL**

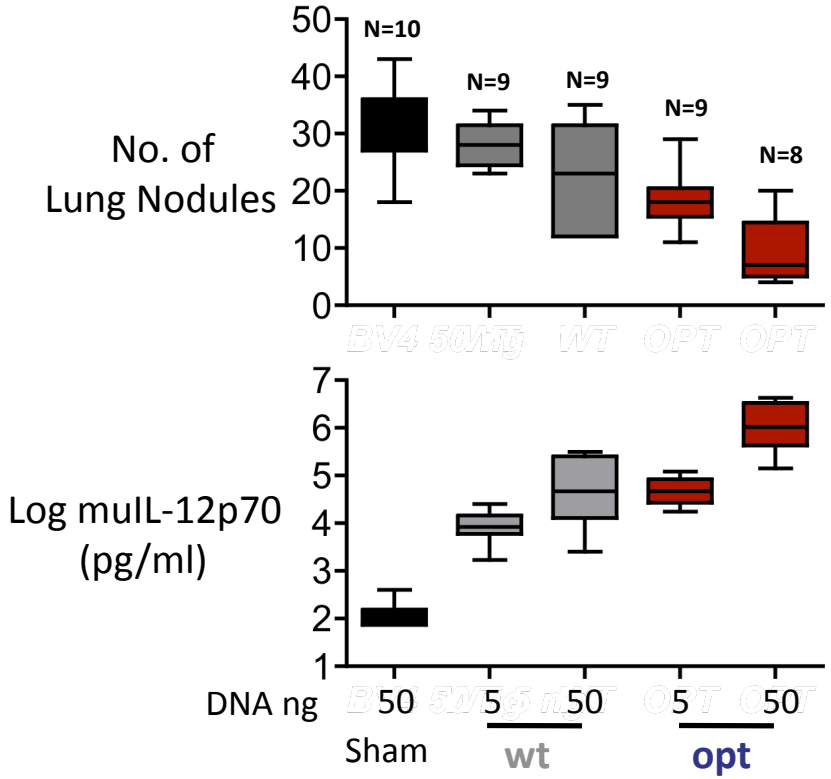
Control

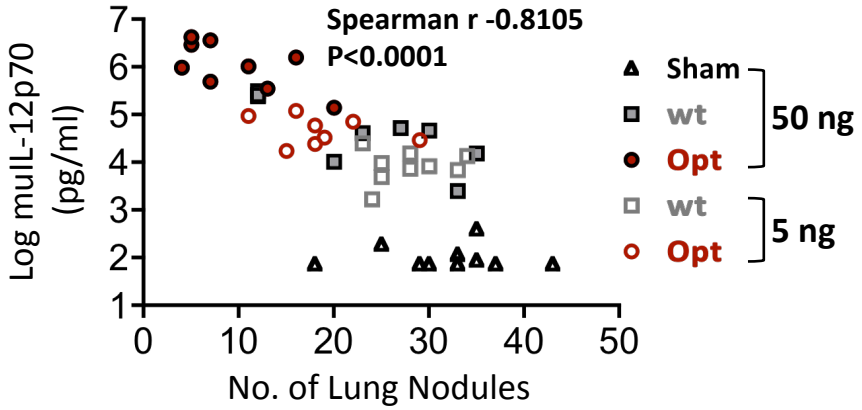


IL-12



Reduced No. of Lung Nodules in the B16 Melanoma Model After hydrodynamic delivery of Optimized Murine IL-12 DNA in Mice





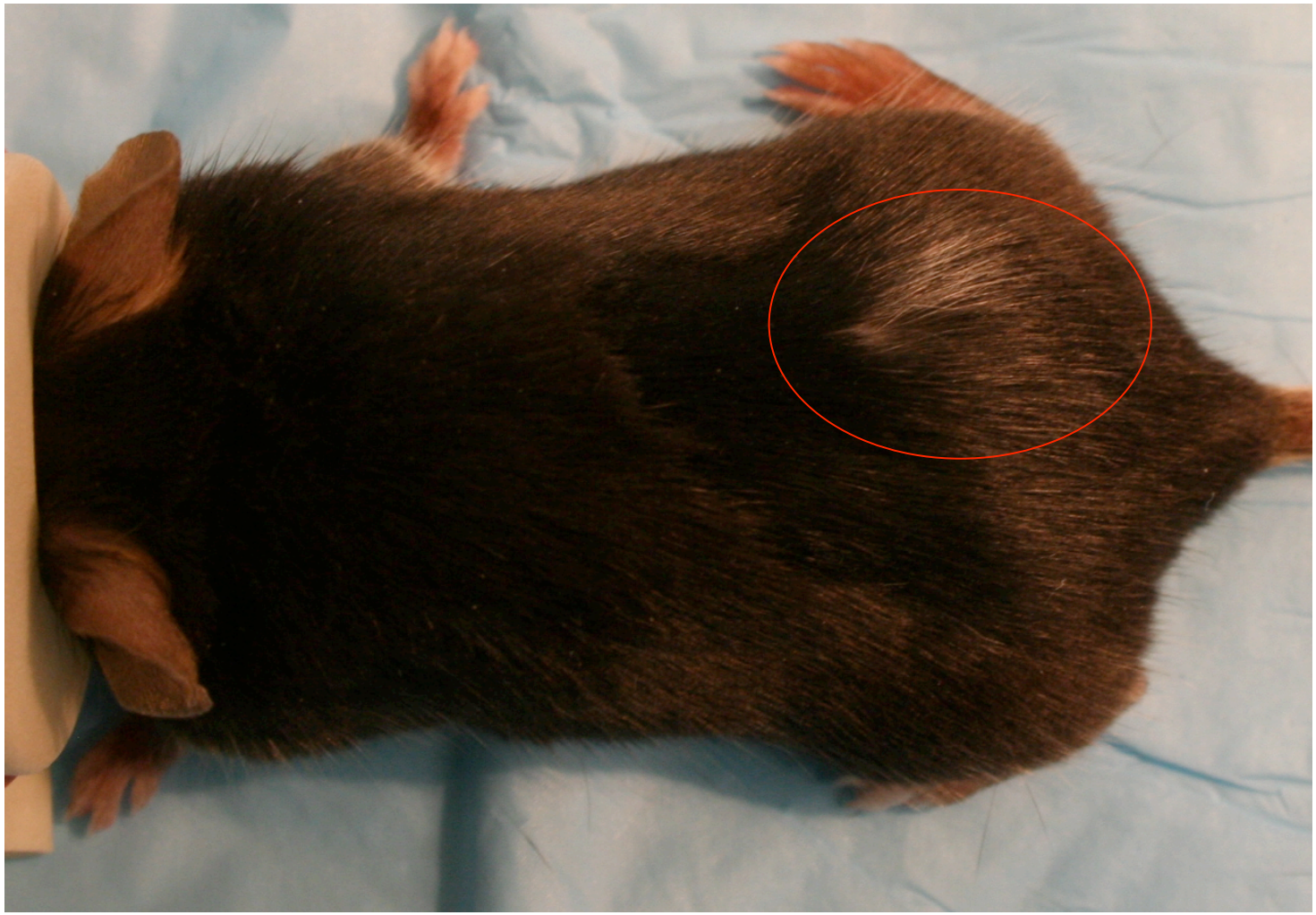
Lung tumor model:

Day 0: intravenous injection of $3x10^5$ B16 Melanoma cells

Day 2: Hydrodynamic delivery of IL-12 DNA (50 and 5 ng of wt and op

Day 22: Sacrifice mice and count lung and liver nodules

Vitiligo In A Mouse Eliminating Large Vascularized B16 Melanoma After IL-12 DNA IT Injection: Suggests Immune Activation Participates In Tumor Remission

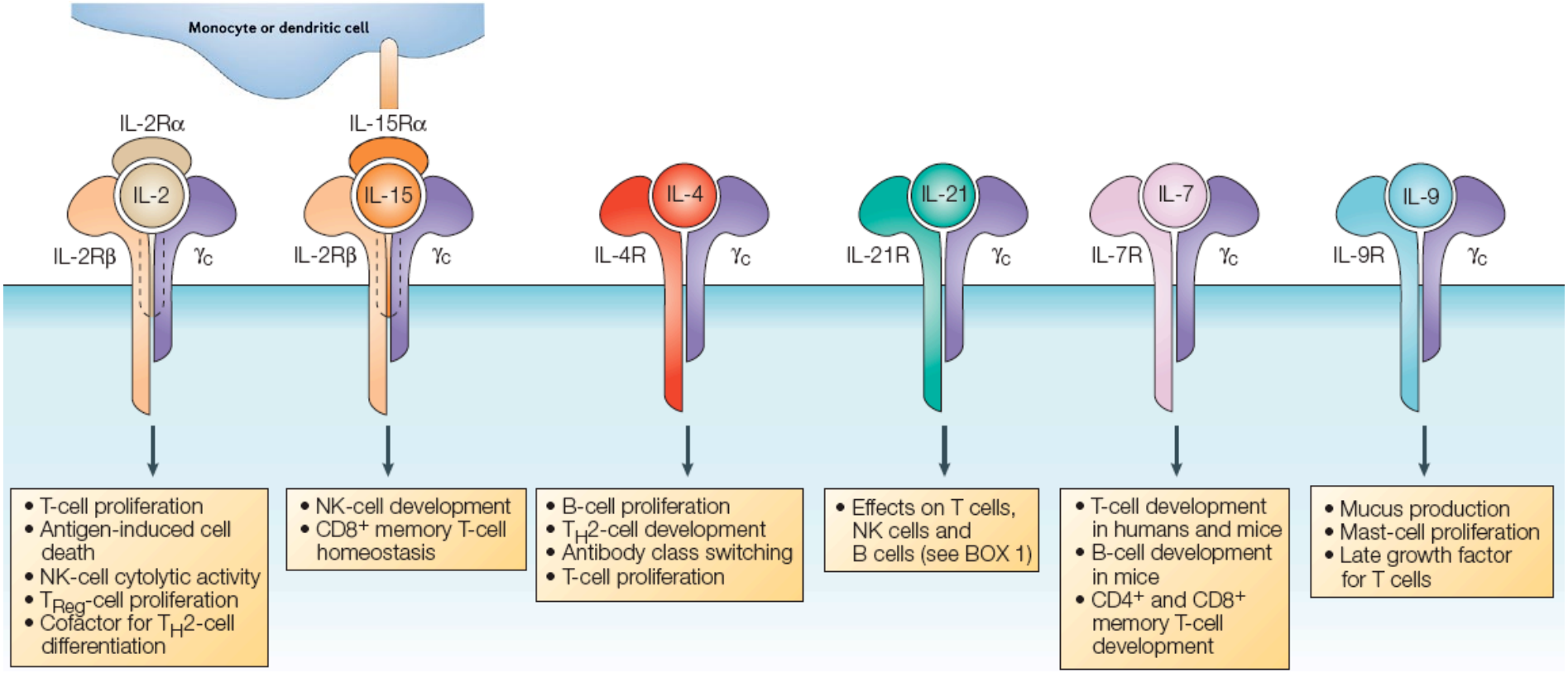


IL-15/IL-15 Receptor alpha Heterodimeric Complexes for Clinical Development

George N Pavlakis

NCI

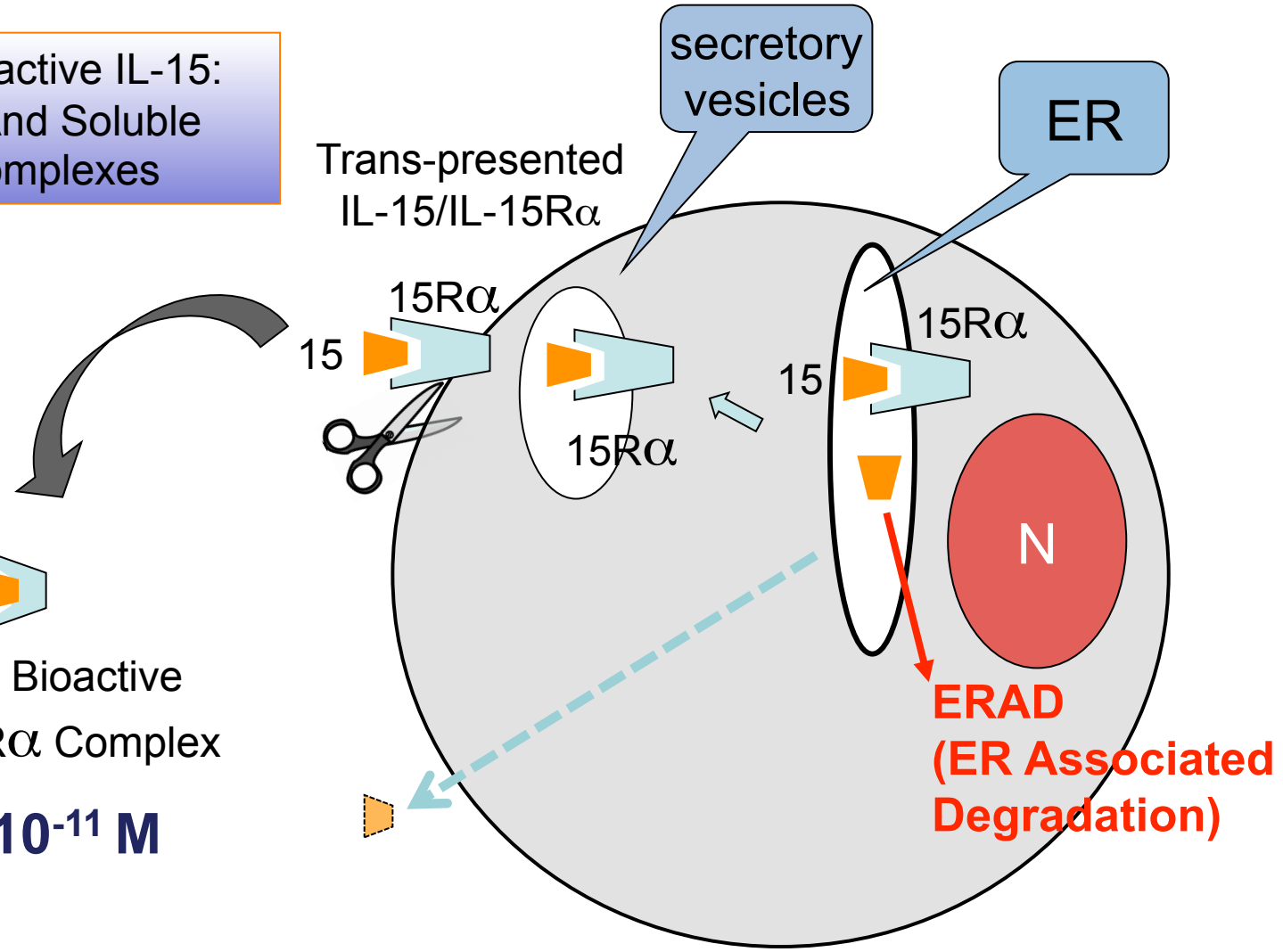
Common γ_c Chain Cytokines



Modified from Waldmann, Nat. Rev. Immunol, (2006)

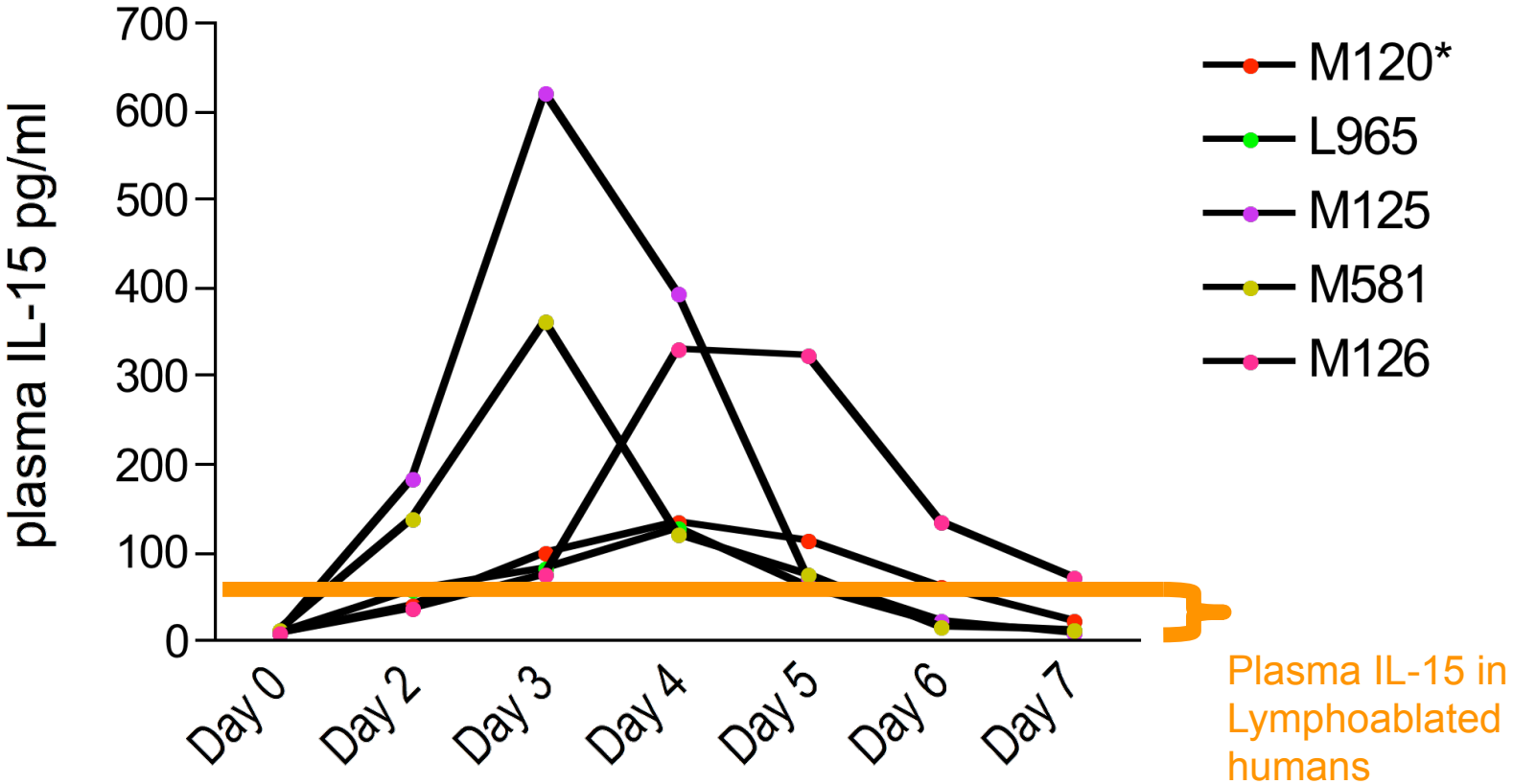
Co-production Leads To Mutual Stabilization of IL-15 and IL-15Rα

Two Forms Of Bioactive IL-15:
Cell Associated And Soluble
IL-15/15Ra Complexes



Bergamaschi, J Biol Chem. 2008 283:4189; J Immunol. 2009 183:3064

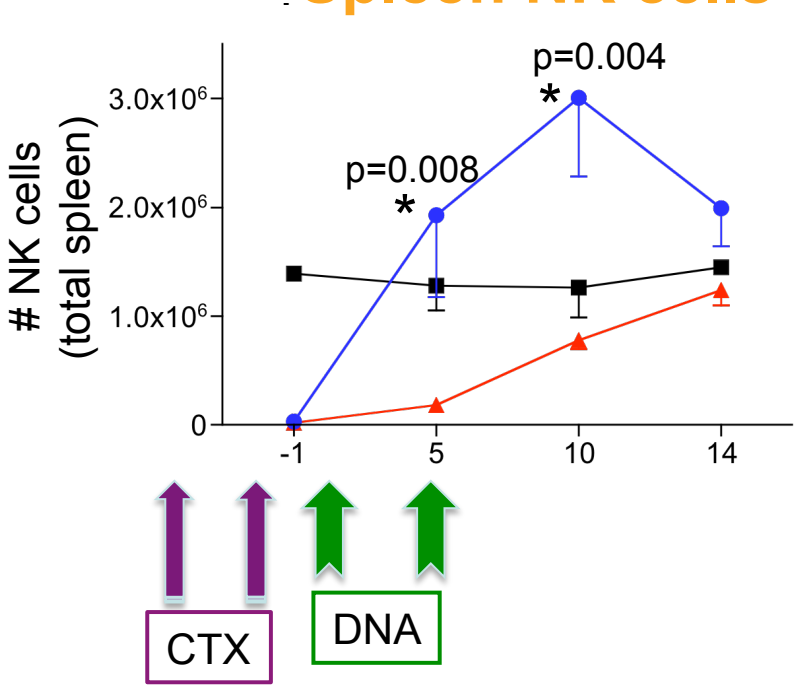
IM Injection of IL-15/15sRa DNA Vectors Leads to Increased Plasma IL-15 Levels



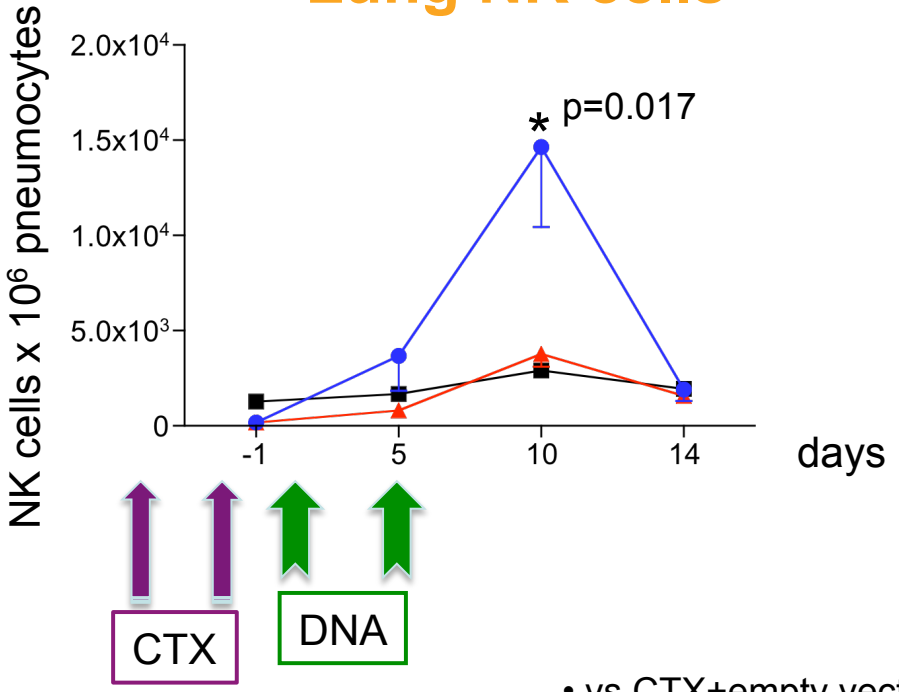
Recovery of NK cells

Control CTX+empty vector CTX+IL-15

Spleen NK cells



Lung NK cells



• vs CTX+empty vector
Non parametric t test

- NK recovery is complete within 14 days after lymphodepleting treatment

- Single administration of IL-15 encoding DNA is sufficient for the complete recovery of NK cells in spleen and lung within

Absolute NK And CD8 Counts In Macaque Plasma During 12 Daily IV Administrations