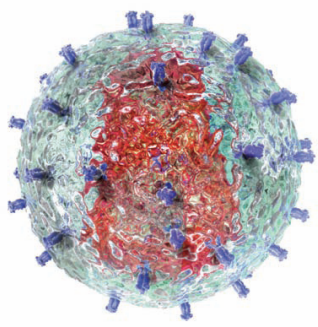


HIV/AIDS: Molecular Biology and pathogenesis

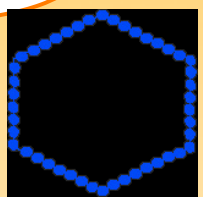
George N. Pavlakis

National Cancer Institute, USA



Infection

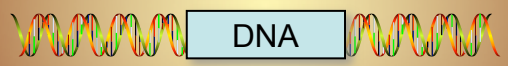
Virion formation



Export, packaging

RNA

Transcription



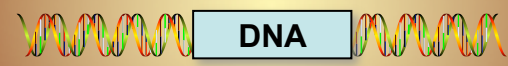
old

Reverse transcription

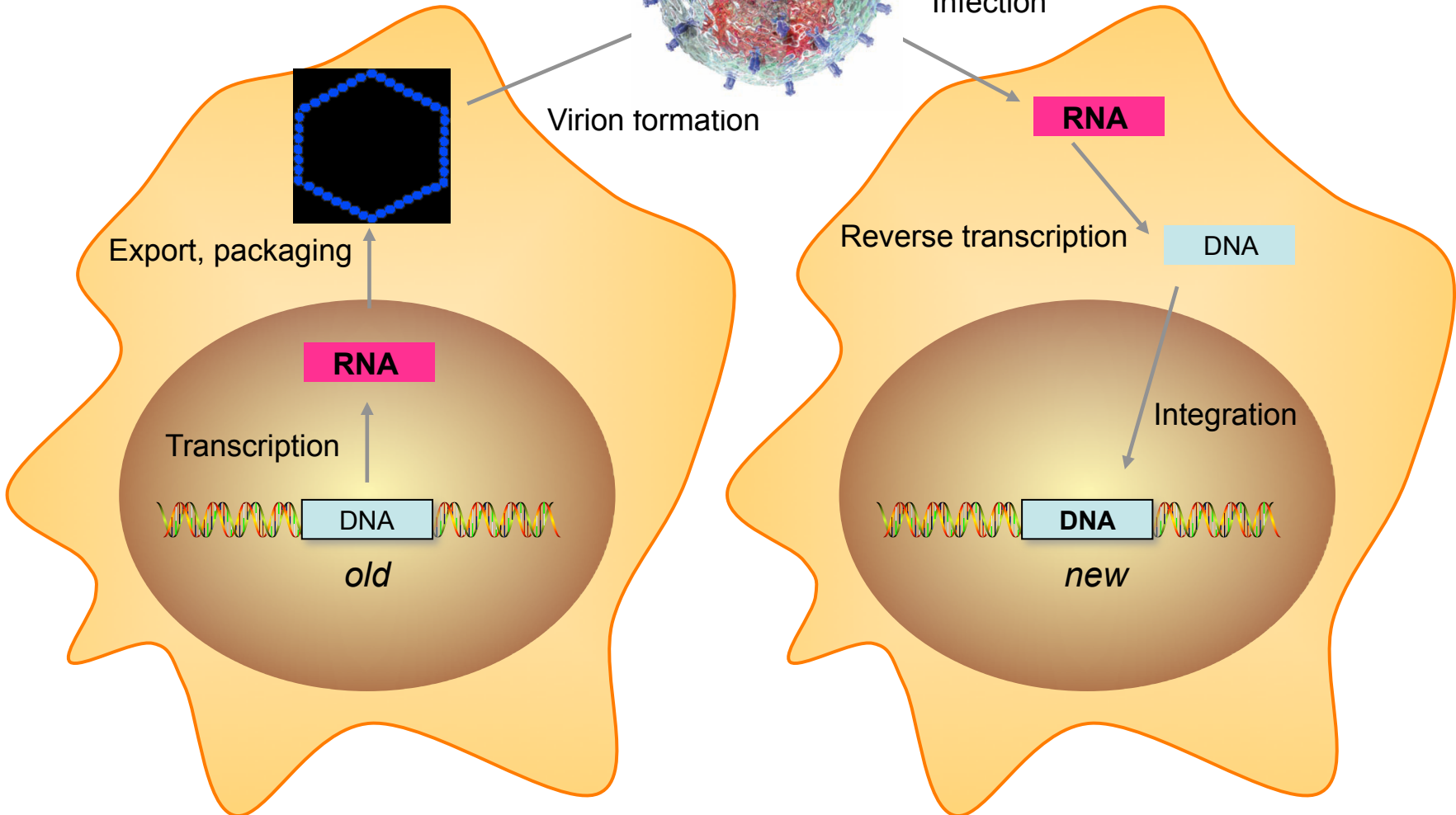
RNA

DNA

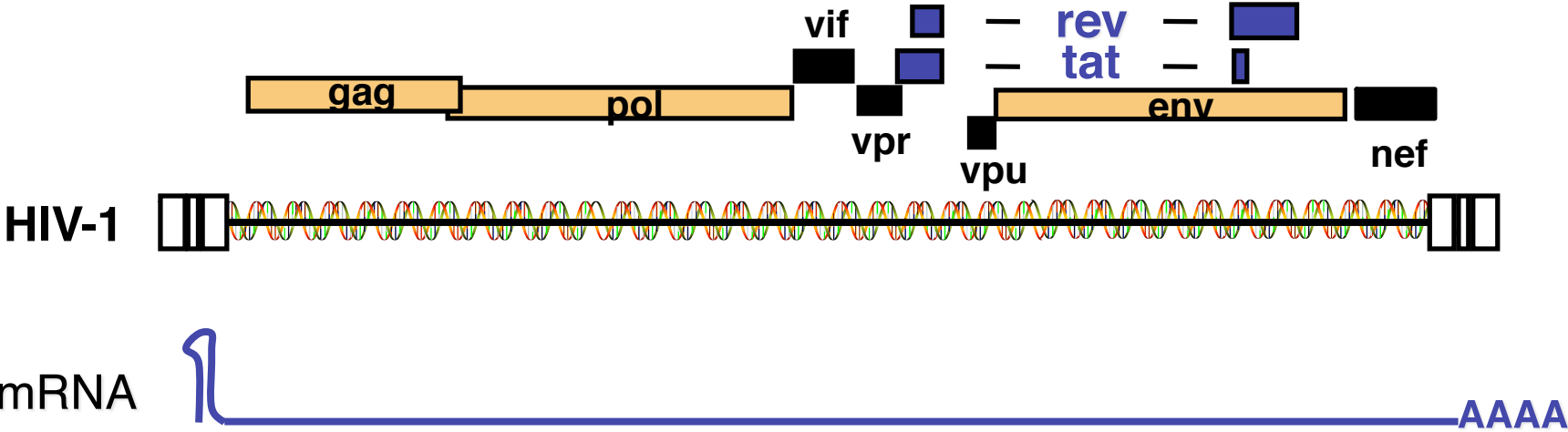
Integration



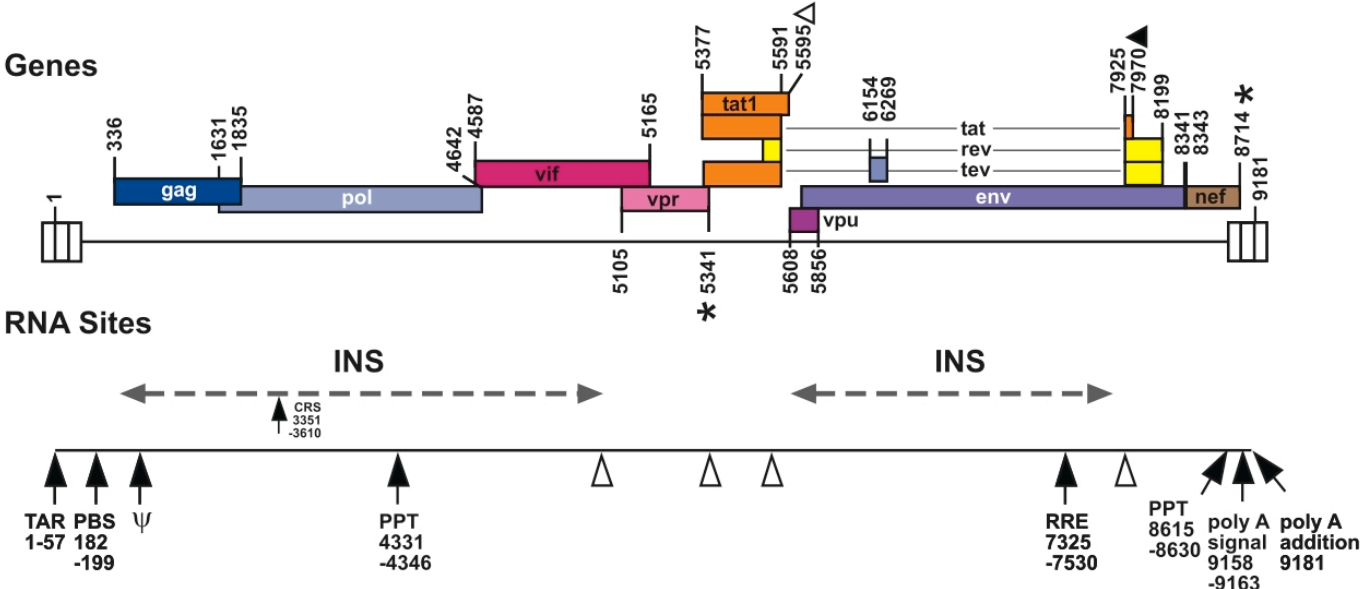
new



Organization Of The HIV-1 Genome



HIV-1 mRNAs

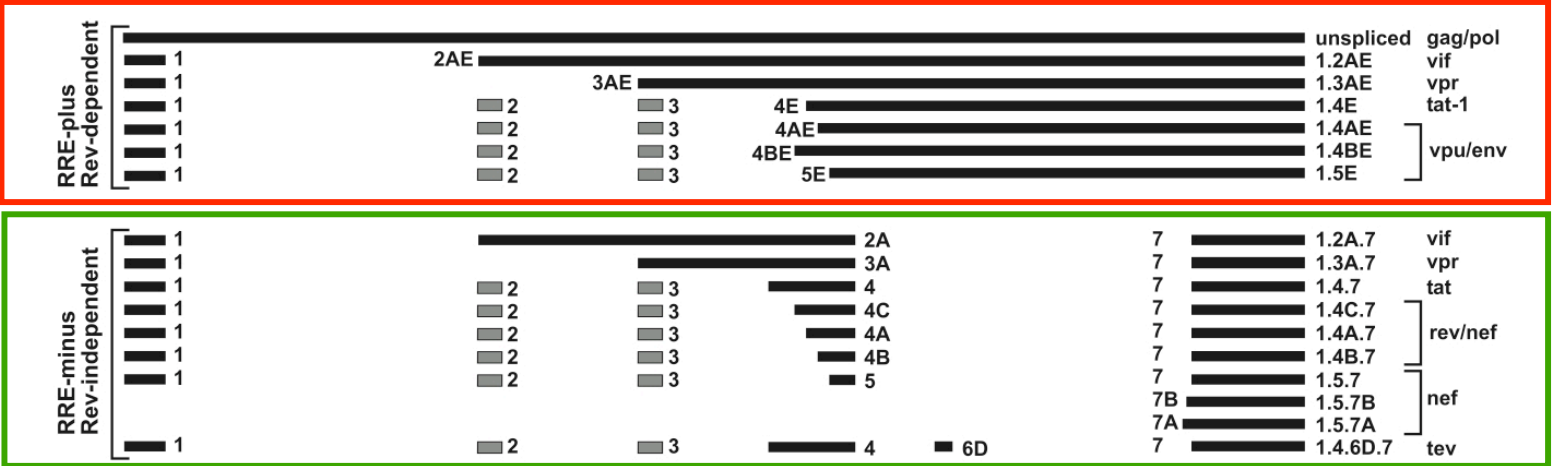


Export mechanism:

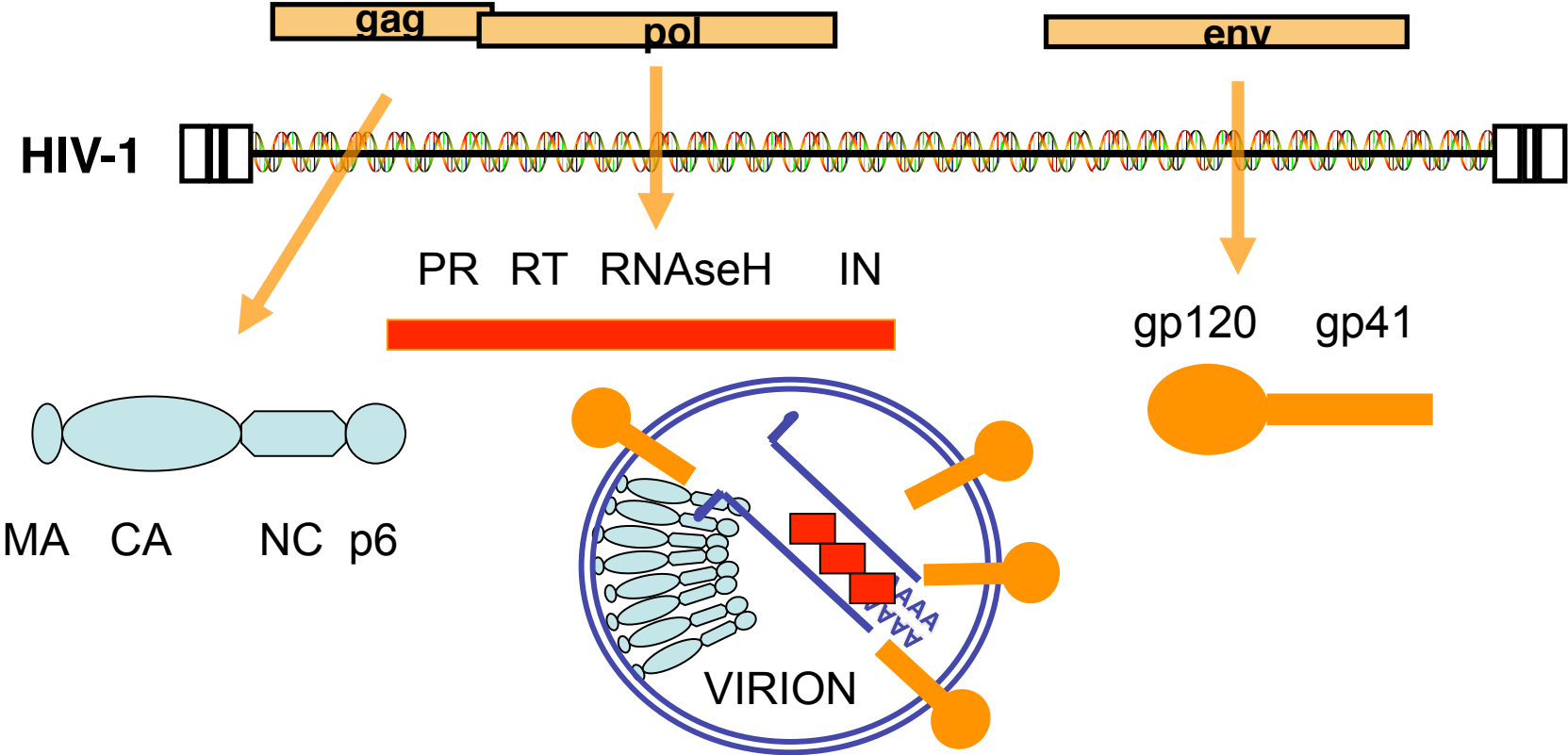
Specialized

Default

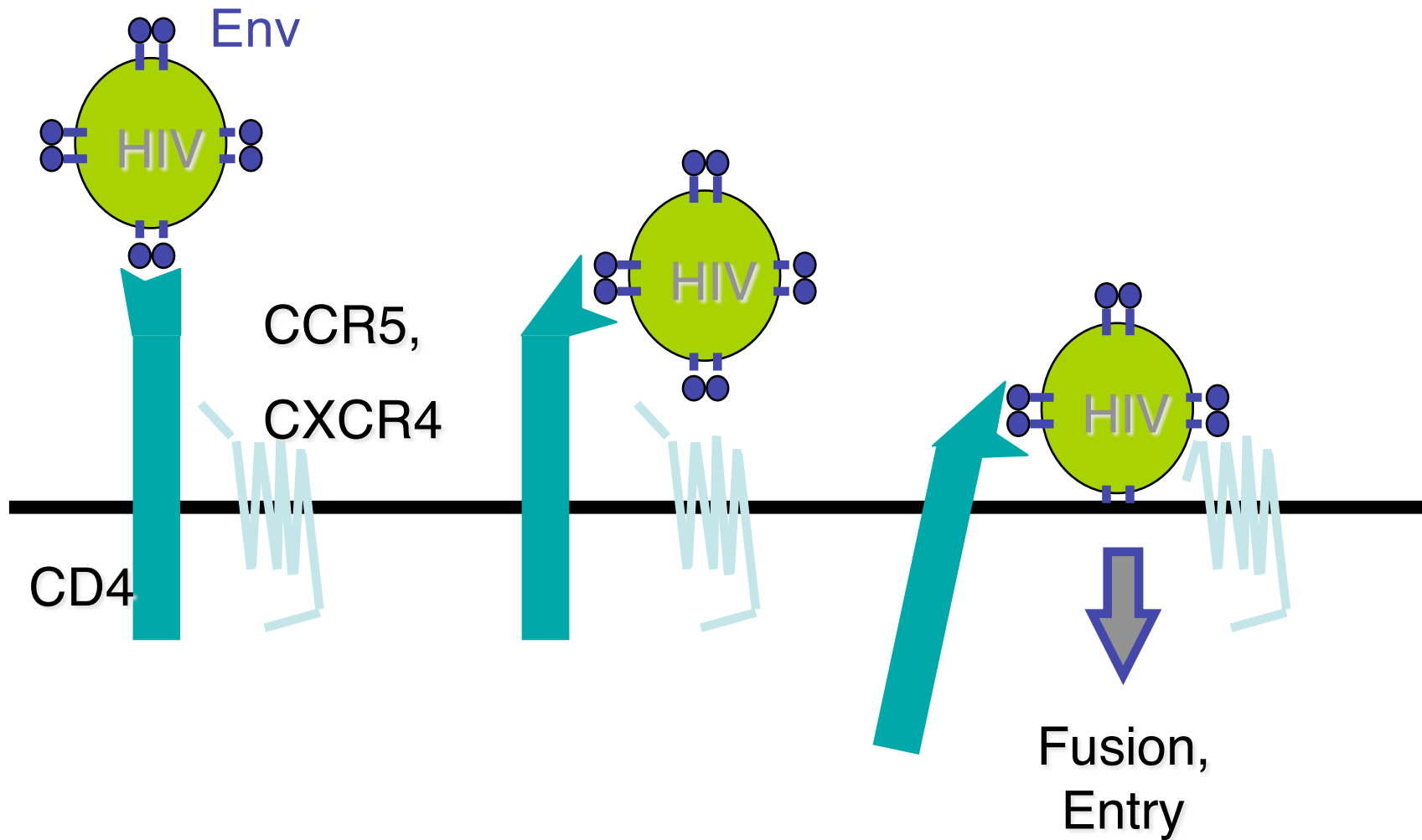
HIV-1 mRNAs



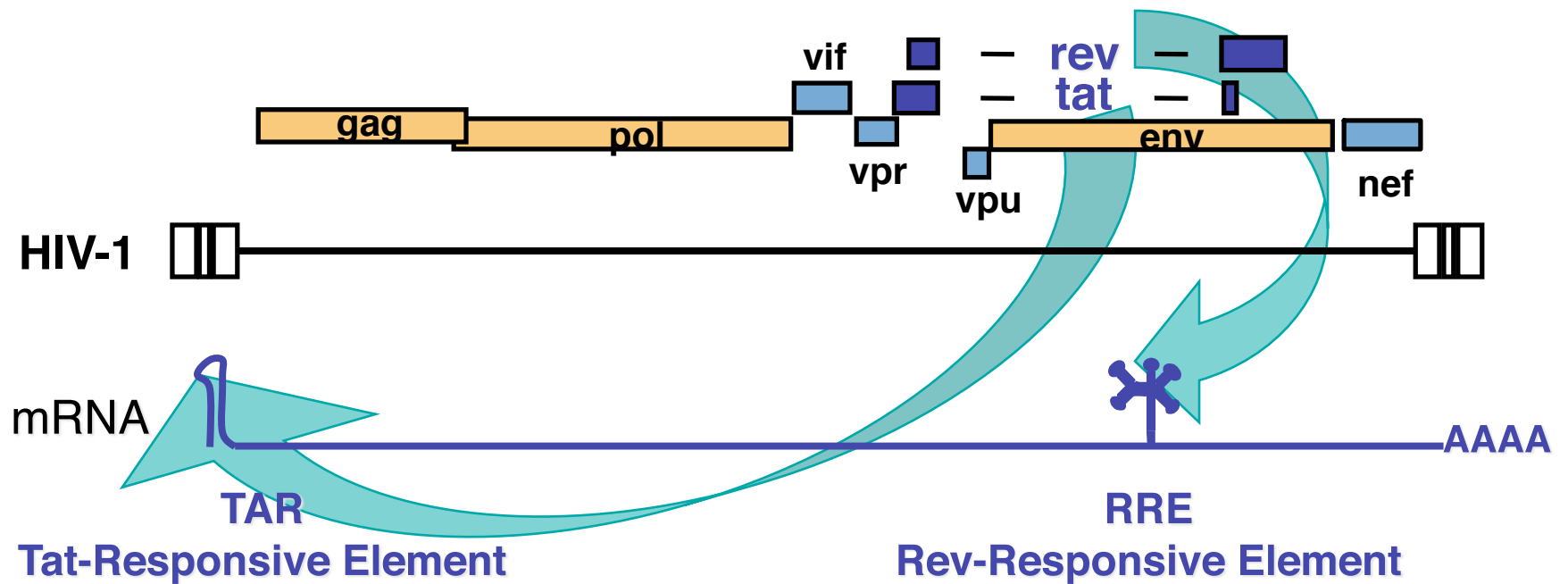
Organization Of The HIV-1 Genome



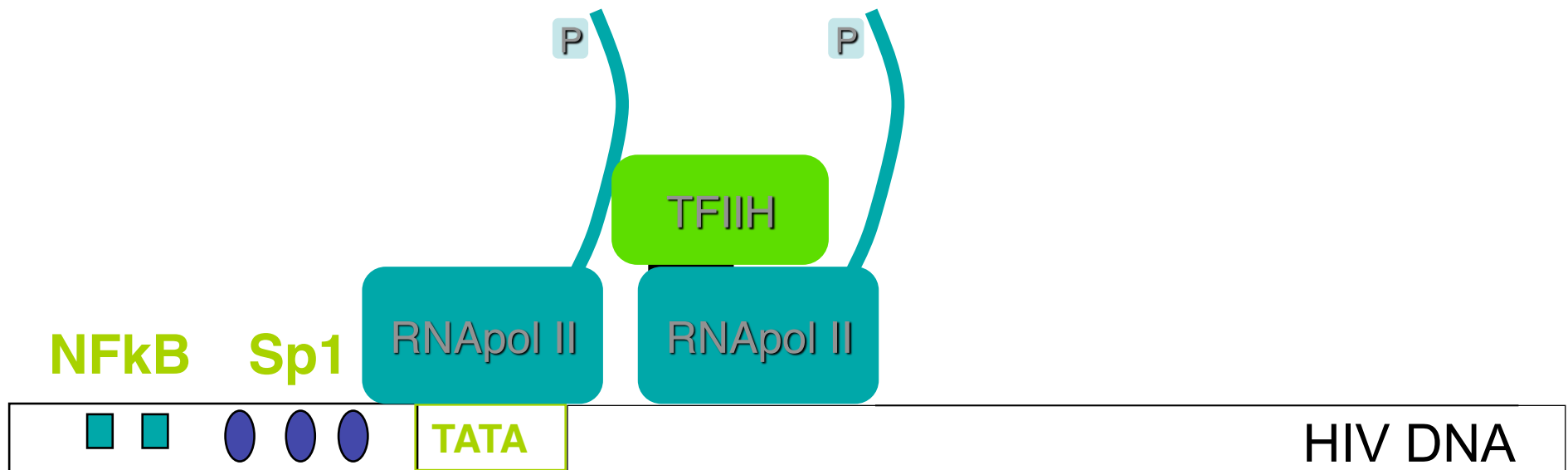
Env And HIV-1 Entry



Tat and Rev Are Essential RNA Binding HIV-1 Proteins



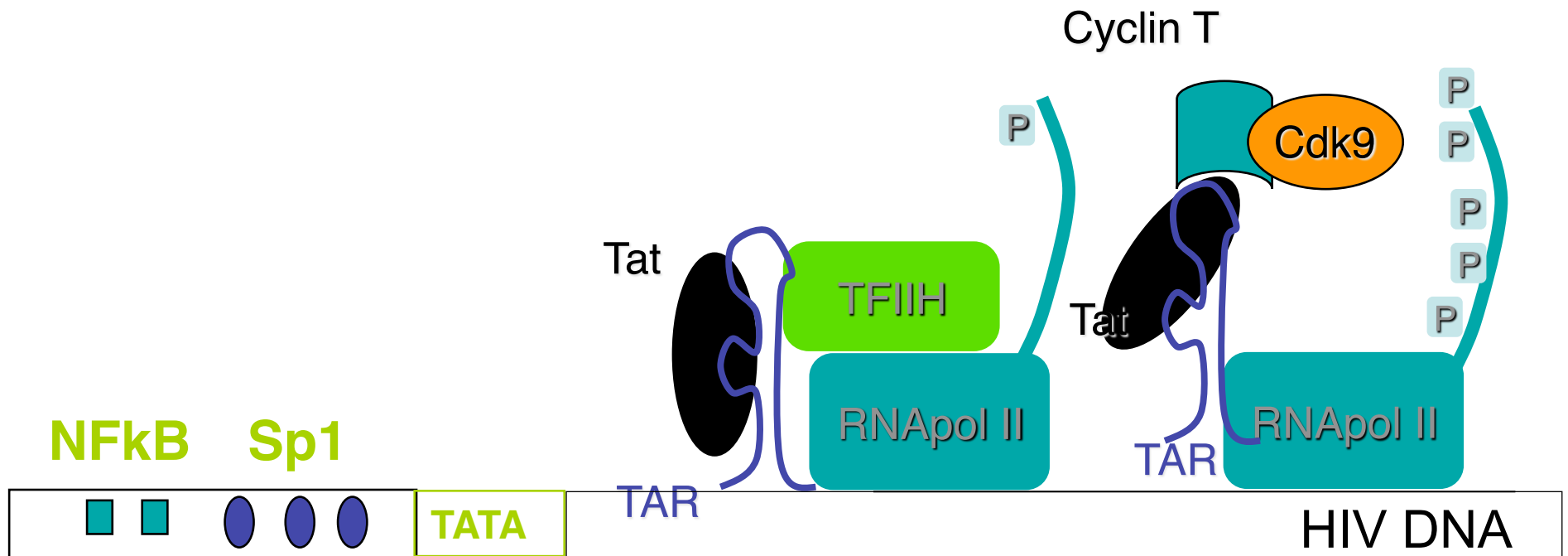
HIV-1 Promoter



Assembly

Clearance

HIV-1 Promoter



Assembly

Clearance

Pausing

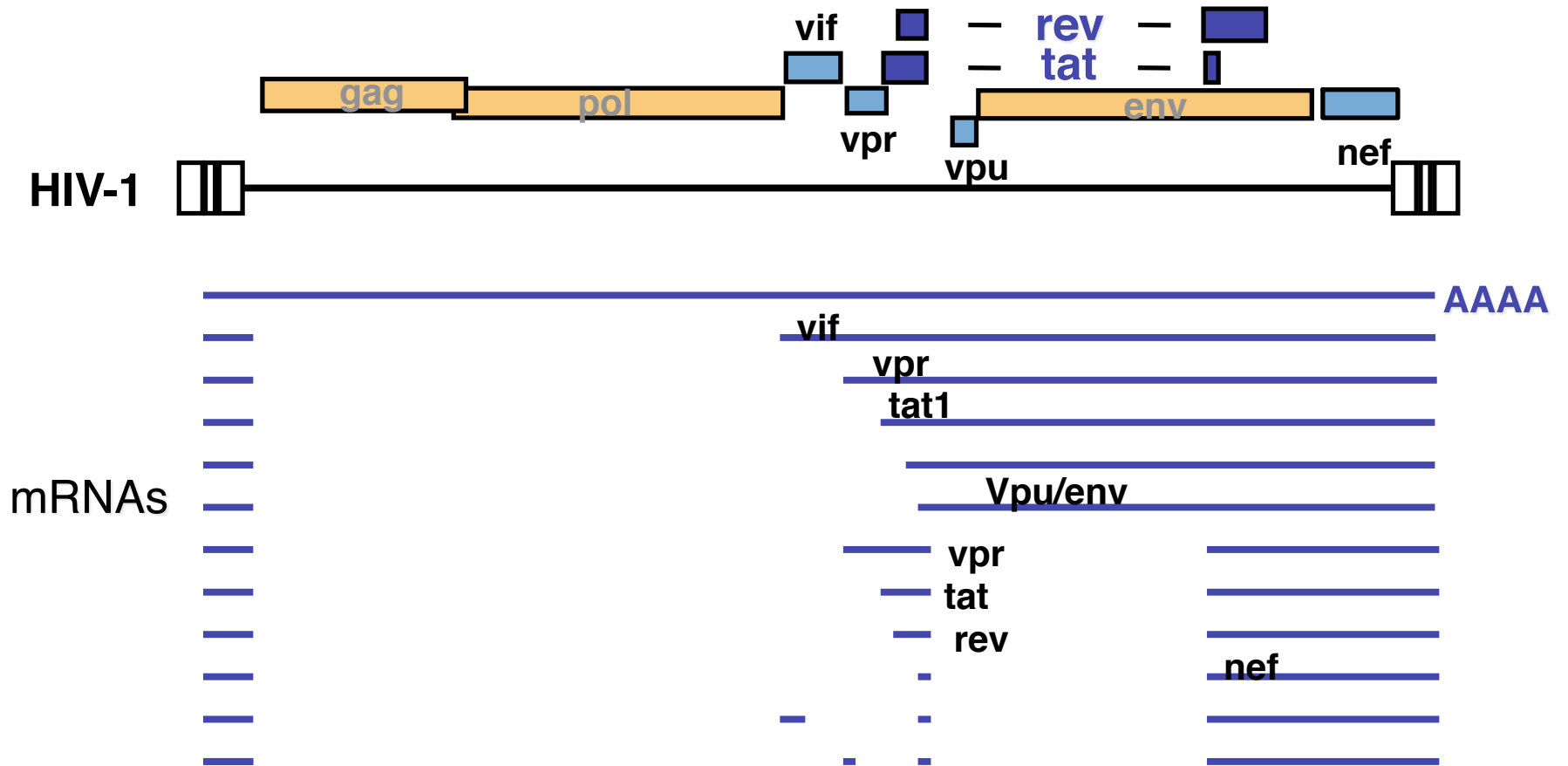
Elongation

HIV-1 mRNA expression

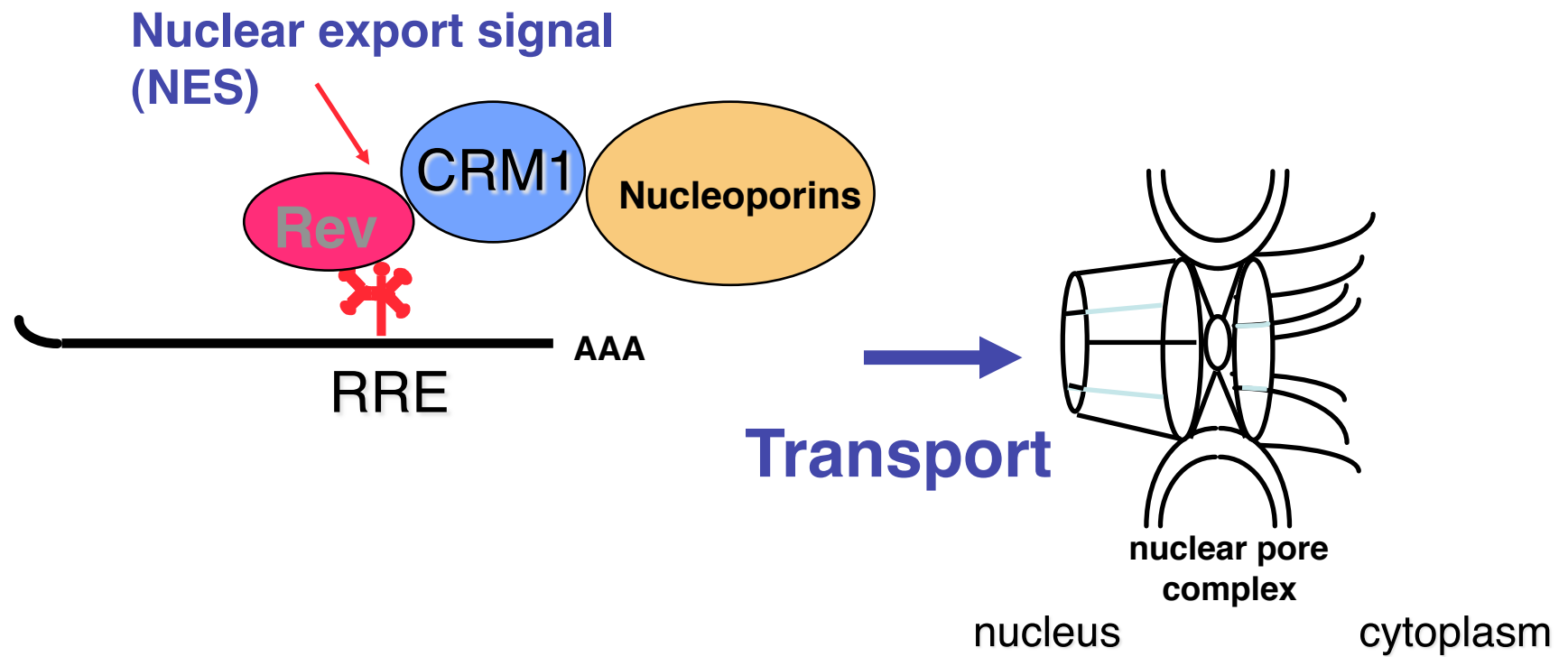
- HIV uses
 - > Alternative splicing
 - > Bicistronic mRNAs
 - > Poly-protein production and processing
 - > frameshifting

to express many proteins from one primary transcript

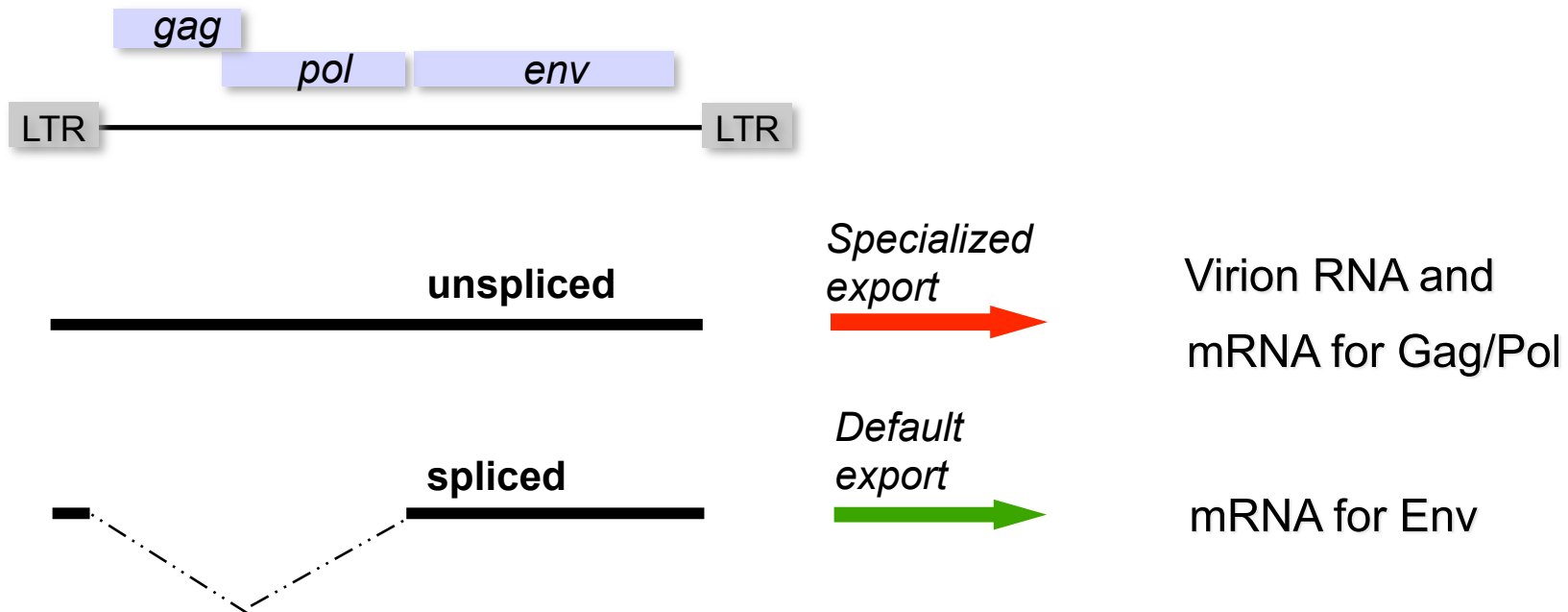
HIV-1 mRNA expression



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

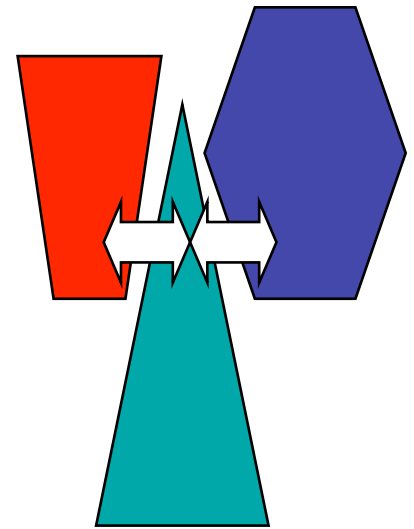
HIV-1 Regulatory and Accessory Proteins

- 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

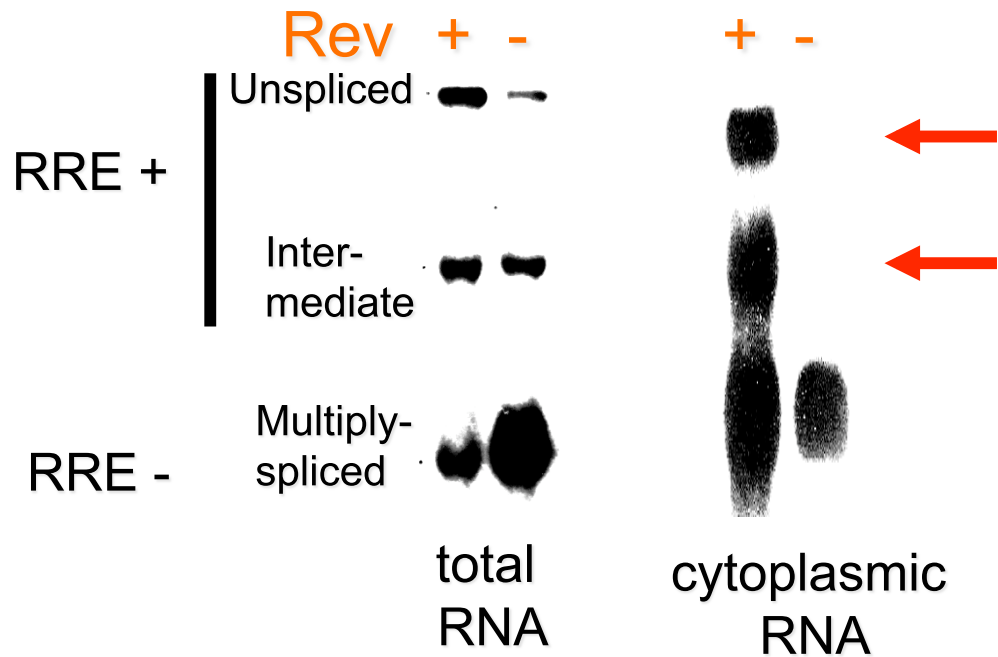
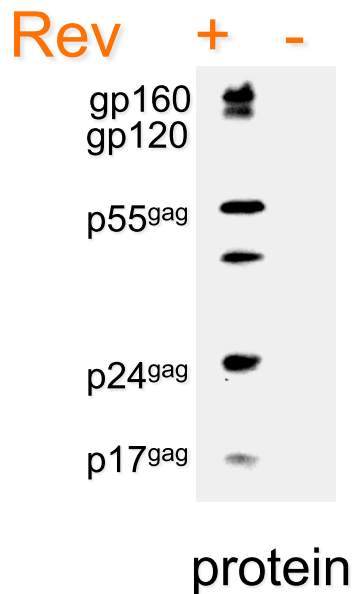
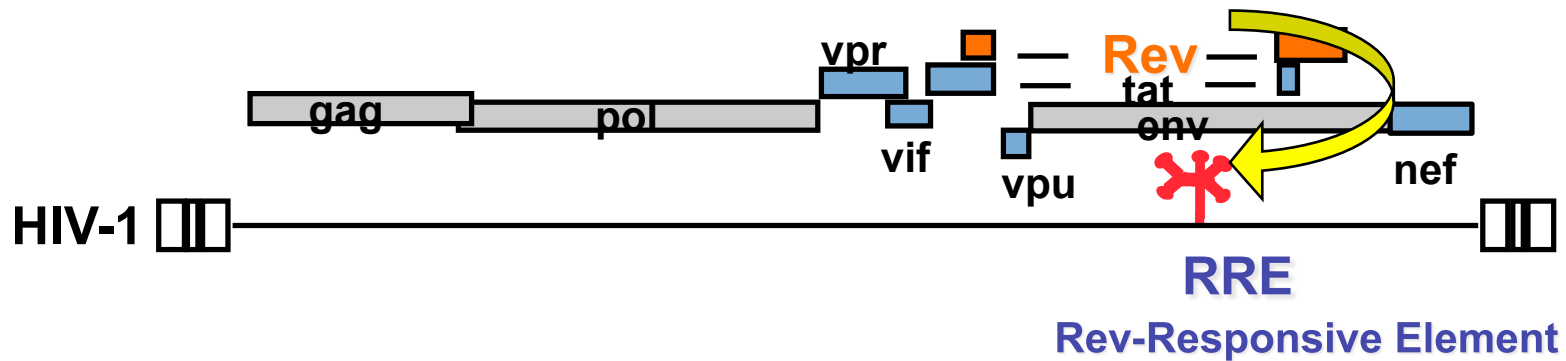
HIV Research Has Benefited Many Fields

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

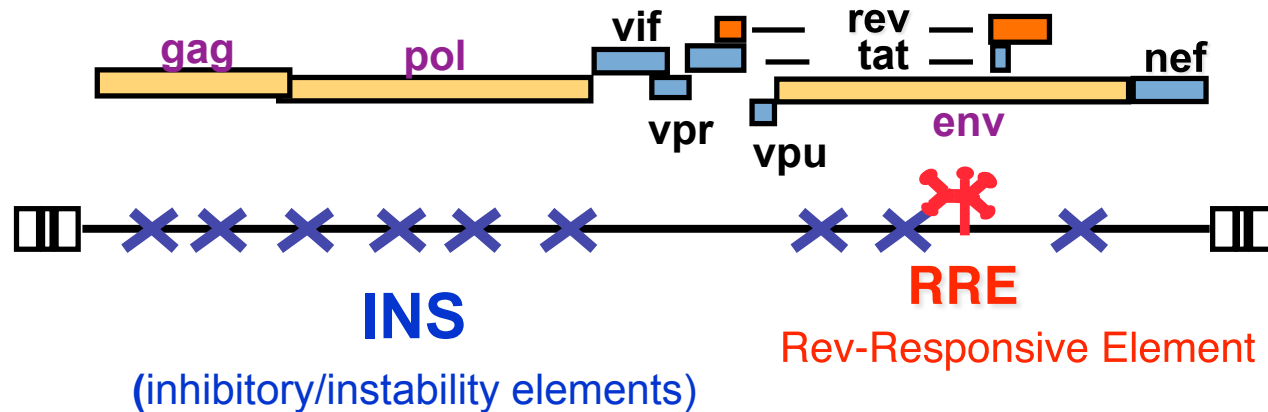
HIV-1 Genome

- 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

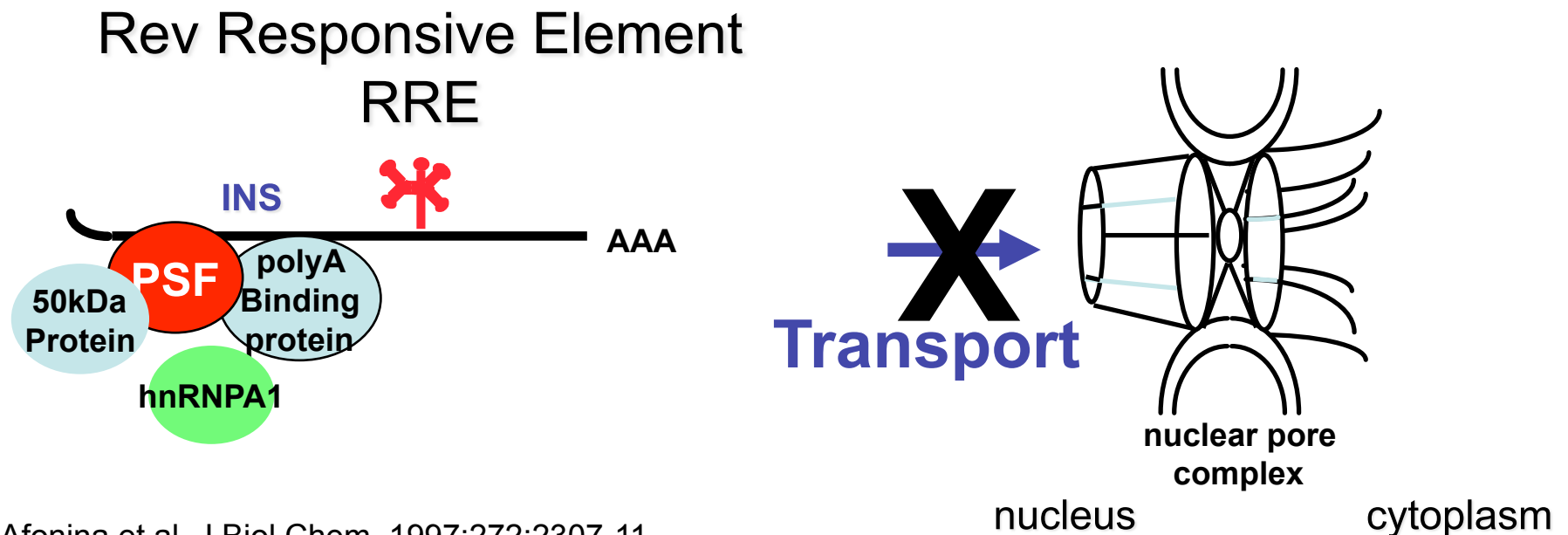


Identification of RNA Elements Responsible for Poor Expression of HIV



- 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

Inhibitory Sequences Interact With Specific Factors In The Nucleus

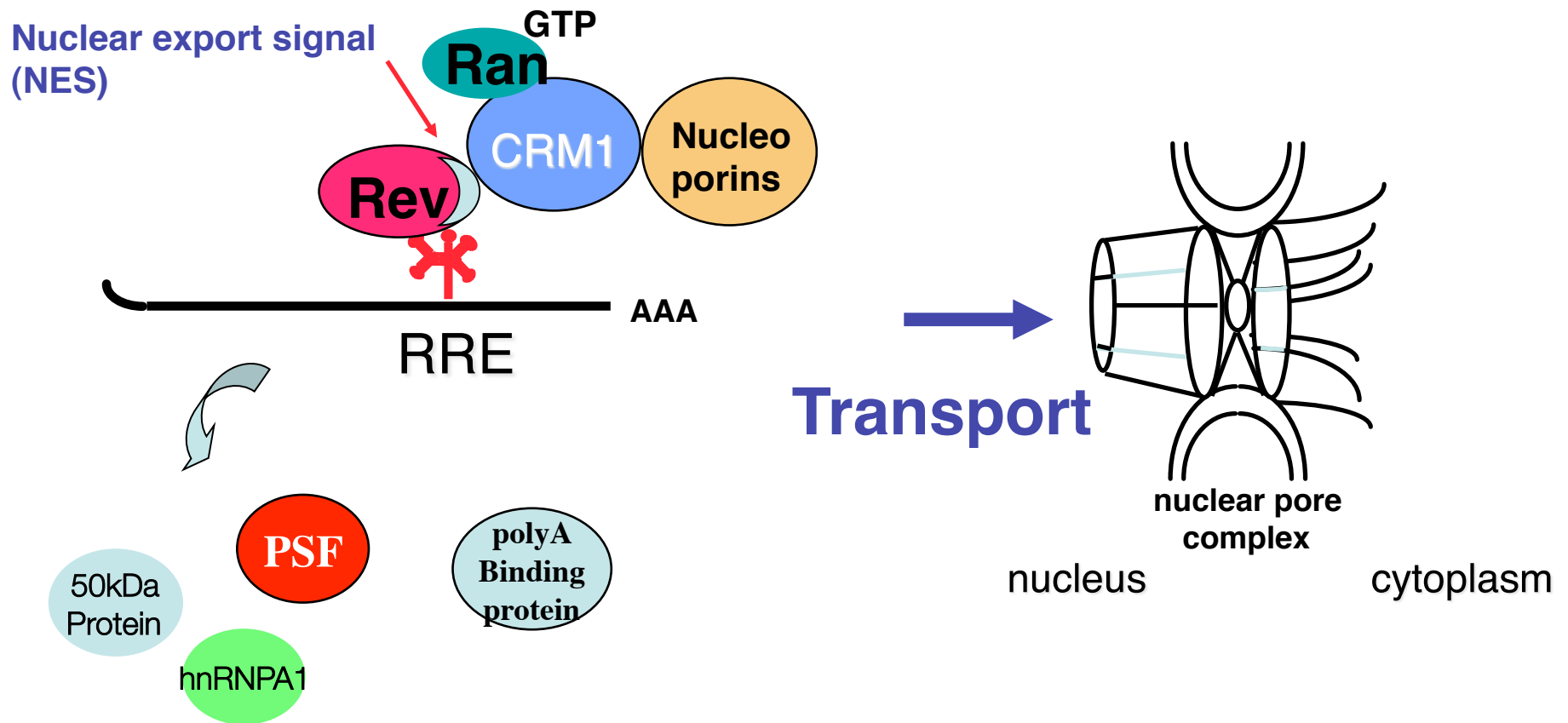


Afonina et al. J Biol Chem. 1997;272:2307-11

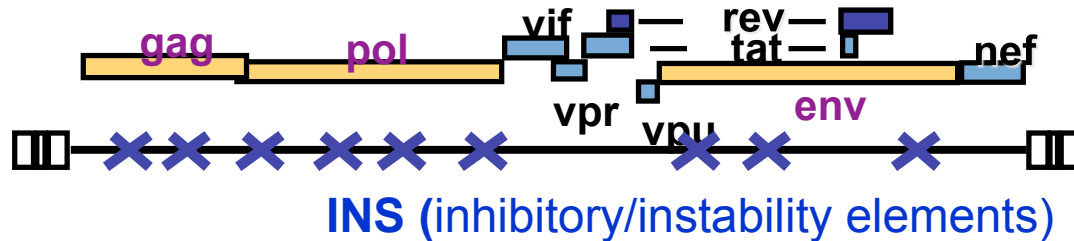
Najera et al J Mol Biol. 1999;285:1951-64

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



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)

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

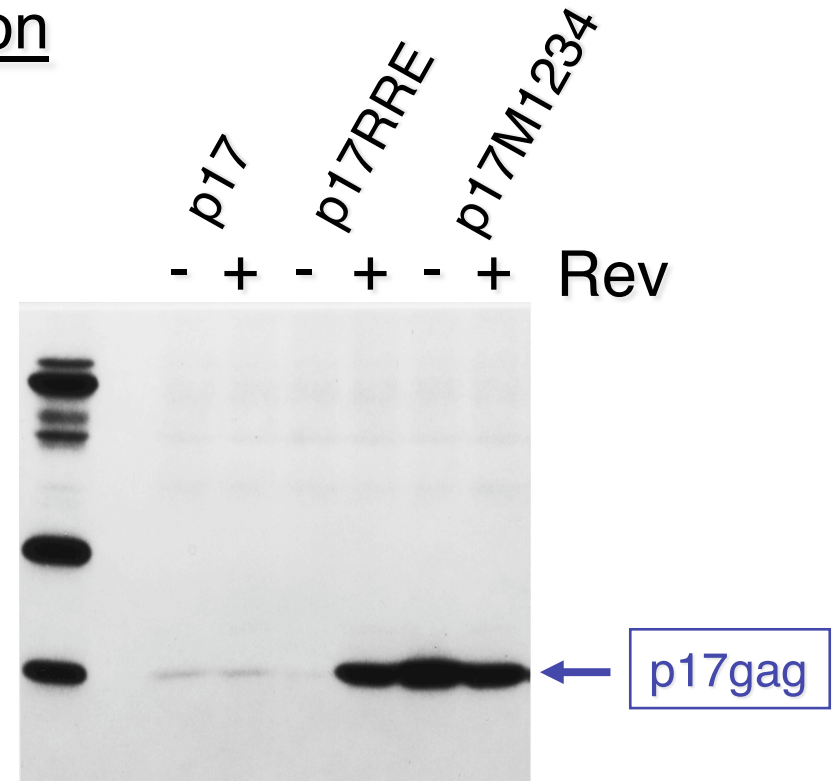
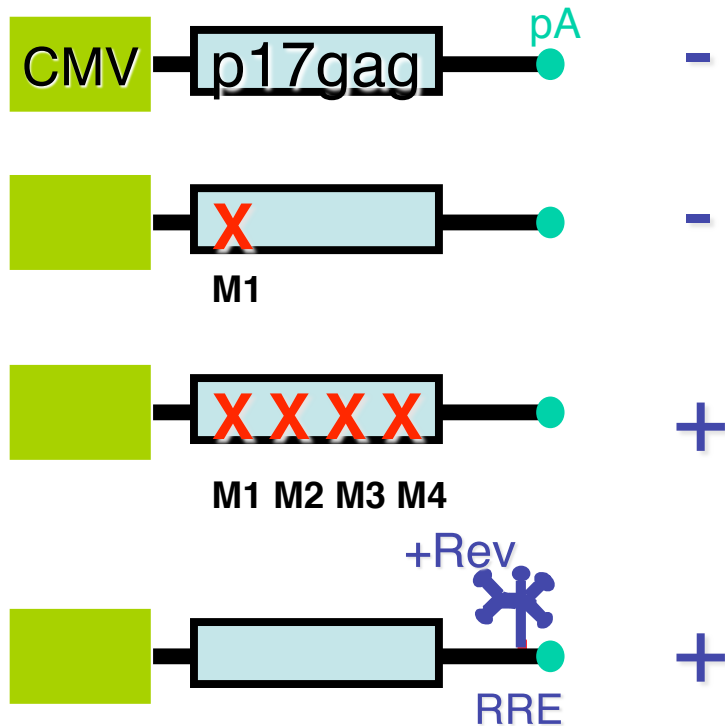
 ...**AAA** **AAA** **TAT** **AAA** **TTA** **AAA** **CAT** **ATA**... . WT

...**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

Gag production



Optimization of HIV-1 gag 94.4% nt identity (84/1503 nt changed)

```

wt atgggtgagagagcgtcagttattaagcgggggagaattagatcgatgggaaaaaattcgg
opt atgggtgagagagcgtcagttattaagcgggggagaattagatcgatgggaaaaaattcgg
*****

wt ttaaggccaggggaaagaaataaaataaaataaaacataatagatgggcaagcagggag
opt ttaaggccaggggaaagaaagatacaagcacaagcacaatcgatgggcaagcagggag
*****
M1

wt ctagaacgattcgcagttaatcctggcctgttagaacaacatcagaaggctgtagacaaata
opt ctagaacgattcgcagttaatcctggcctgttagaacaacatcagaaggctgtagacaaata
*****

wt ctgggacagctacaaccatcccttcagacagggatcagaagaacttagatcattataaat
opt ctgggacagctacaaccatcccttcagacagggatcagaagaacttagatcattatacaac
*****
M2

wt acagtagcaaccctctattgtgtgcatcaaggatagagataaaagacaccaaggaagct
opt acagtagcaaccctctattgtgtgcatcaaggatagagataaaagacaccaaggaagct
*****
M3

wt ttagacaagatagaggaaagagcaaaacaaagtaagaaagagcagcaagcagcagct
opt ttagacaagatagaggaaagagcaaaacaaagtaagaaagagcagcaagcagcagct
*****
M4

wt gacacaggacacagcaatcaggtcagccaaaattaccctatagtgacagaacatccagggg
opt gacacaggacacagcaatcaggtcagccaaaattaccctatagtgacagaacatccagggg
*****

wt caaatggtacatcaggccatatacctagaactttaaatgcatgggtaaaagttagtagaa
opt caaatggtacatcaggccatatacctagaactttaaatgcatgggtaaaagttagtagaa
*****

wt gagaaggctttcagcccagaagtgtataccatgttttcagcattatcagaaggagccacc
opt gagaaggctttcagcccagaagtgtataccatgttttcagcattatcagaaggagccacc
*****

wt ccacaagatttaaacaccatgctaaacacagtggggggacatcaagcagccatgcaaatg
opt ccacaagaccttaaacaccatgctaaacacagtggggggacatcaagcagccatgcaaatg
*****
M6

wt ttaaaaagagaccatcaatgaggaagctgcagaatgggatagagtgcatccagtgcatgca
opt ttaaaaagagaccatcaatgaggaagctgcagaatgggatagagtgcatccagtgcatgca
*****

wt gggcctattgcaccaggccagatgagagaaaccaaggggaaagtgcatagcaggaactact
opt gggcctattgcaccaggccagatgagagaaaccaaggggaaagtgcatagcaggaactact
*****

wt agtacccttcaggaacaaataggatggatgacaaaataatccacctatcccagtaggaga
opt agtacccttcaggaacaaataggatggatgacaaaataatccacctatcccagtaggaga
*****

```

```

wt atctcaaaagatggataatcctgggattaaataaaatagtaagaatgtatagccctacc
opt atctcaaaagatggataatcctgggattaaataaaatagtaagaatgtatagccctacc
*****
M7 M8

wt agcattctggacataagacaaggaccaaaagaacccttagagactatgtagaccgggttc
opt agcattctggacataagacaaggaccaaaagaacccttagagactatgtagaccgggttc
*****

wt tataaaactctaagagcagcaagcttcacaggaggtaaaaaattggatgacagaacc
opt tataaaactctaagagcagcaagcttcacaggaggtaaaaaattggatgacagaacc
*****

wt ttgttggtccaaaatgcaaccagattgtaagactatffffaaahgcatgggaccagcg
opt ttgttggtccaaaatgcaaccagattgtaagactatccttaaggtcctcggccagcg
*****
M10

wt gctacactagaagaaatgatgacagcatgtcagggagtaggaggaccggccataaggca
opt gctacactagaagaaatgatgacagcatgtcagggagtaggaggaccggccataaggca
*****

wt agagttttggctgaagcaatgagccaaatgtaacaaatcagctaccataatgatgcagaga
opt agagttttggctgaagcaatgagccaaatgtaacaaatcagctaccataatgatgcagaga
*****
M11

wt ggcaatttttaggaacaaagaaagattgttaagtgttcaattgtggcaaaagaagggcac
opt ggcaatttttaggaacaaagaaagattgttaagtgttcaattgtggcaaaagaagggcac
*****
M12

wt acagccagaaatgcaagggccccaggaagaaagggctgttggaaatgtgaaaggaagga
opt acagccagaaatgcaagggccccaggaagaaagggctgttggaaatgtgaaaggaagga
*****
M13

wt caccaaatgaaagattgtactgagagacaggctaatttttagggaagatctggccttcc
opt caccaaatgaaagattgtactgagagacaggctaatttttagggaagatctggccttcc
*****

wt tacaaggggaagggcagggaaatctcttcagagcagaccagagccaacagccccaccagaa
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*****

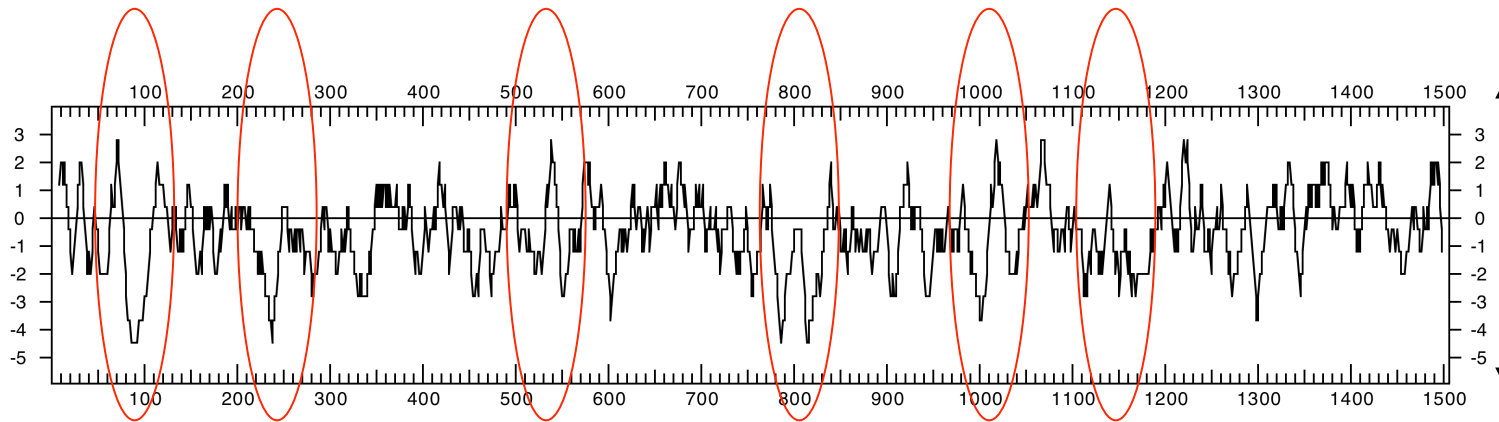
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opt gagagcttcaggtctggggtagagacaacaactccccctcagaagcaggagccgatagac
*****

wt aaggaactgtatcctttaactccctcagatcactctttggcaacgaccctcgtcacaa
opt aaggaactgtatcctttaactccctcagatcactctttggcaacgaccctcgtcacaa
*****

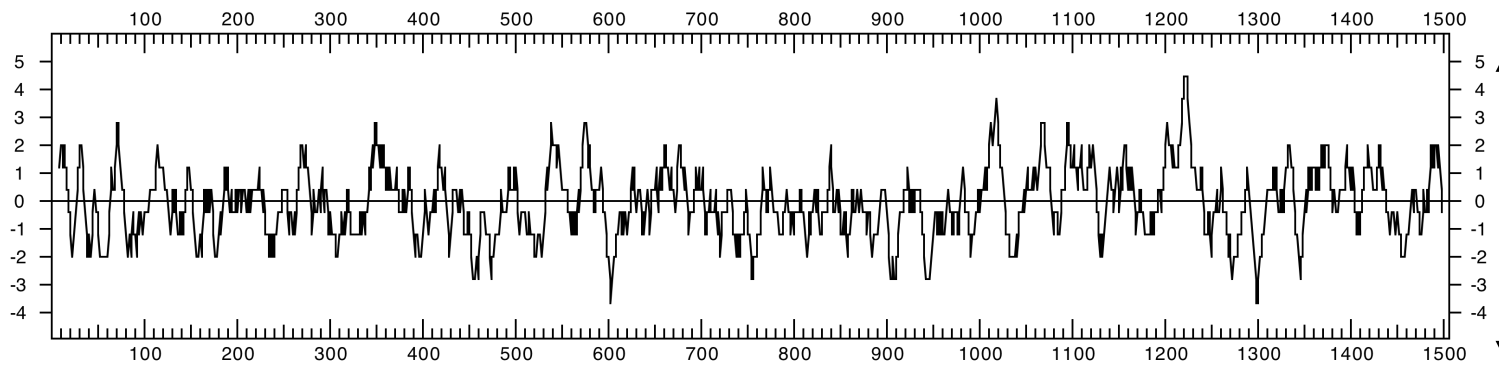
wt taa
opt taa
***

```

AU- and GC-profile of HIV gag



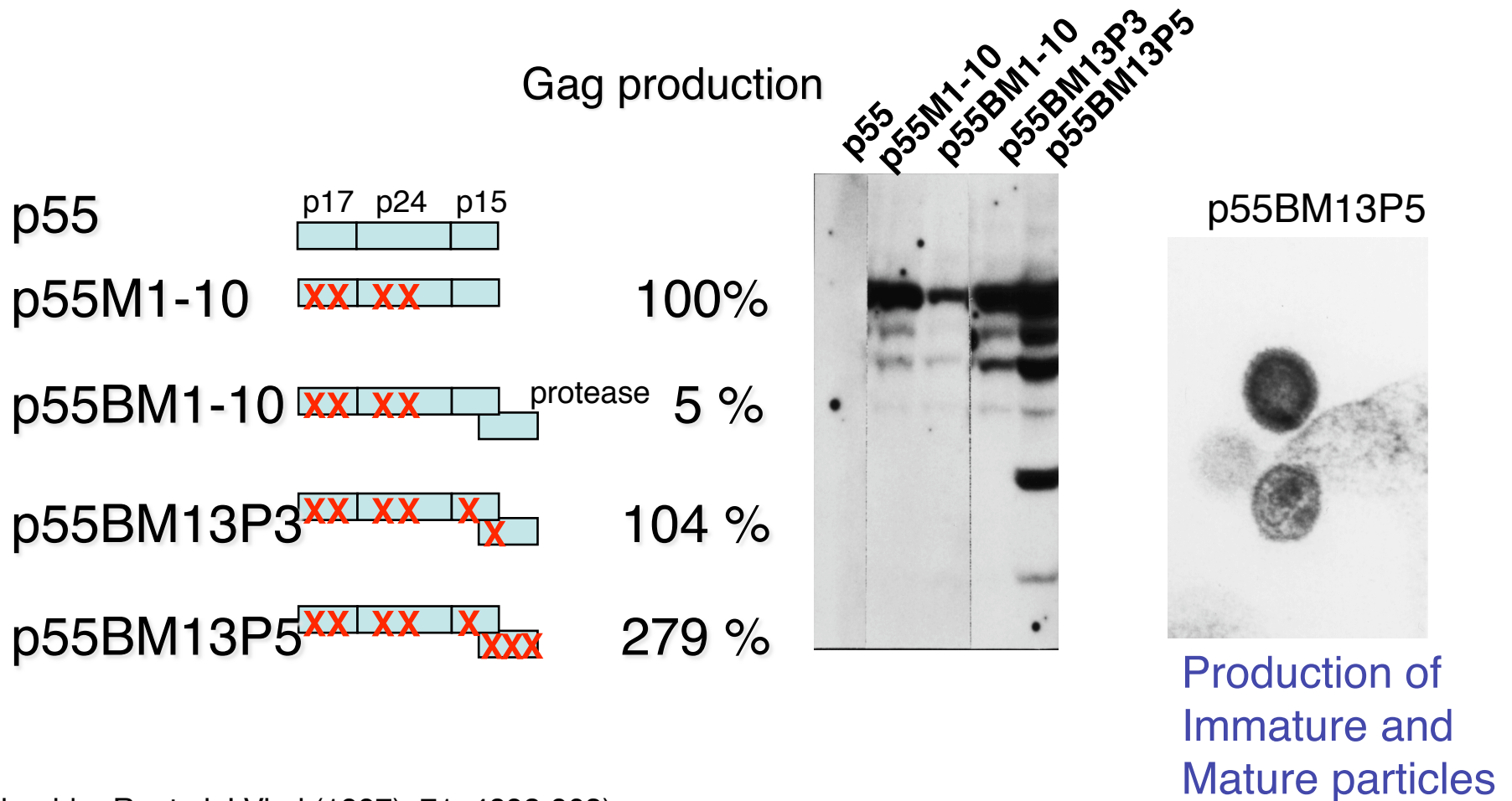
WT
GC = 43.9%
AU = 56 %



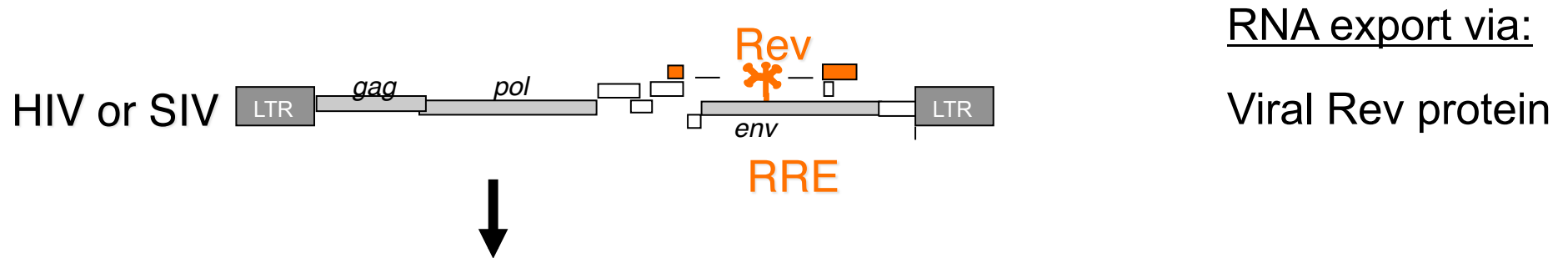
OPT
GC = 48.9%
AU = 51 %

Optimized gag mRNA has increased GC-content

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



Posttranscriptional Regulation Is Essential for HIV Expression



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

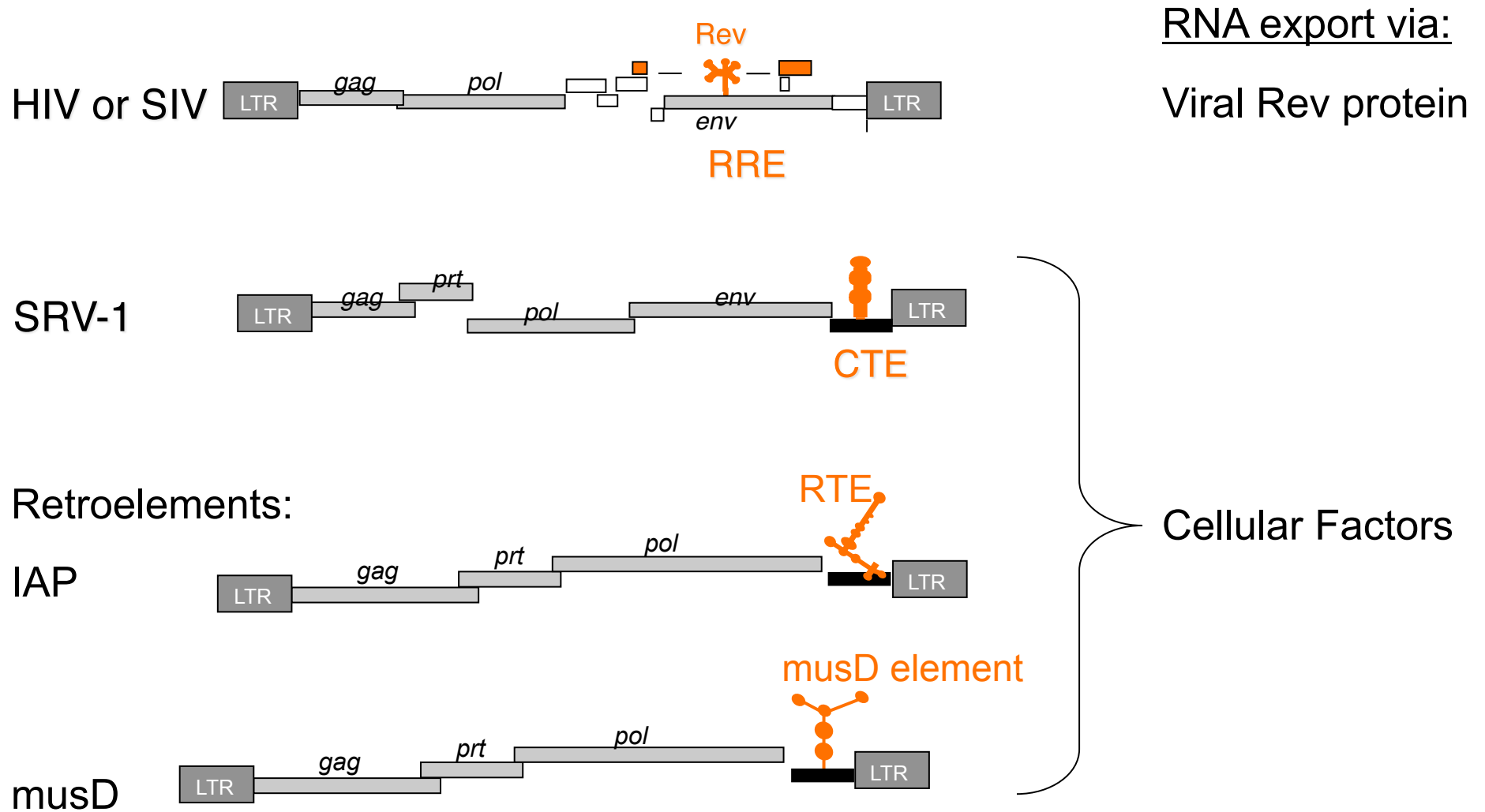
- model to study mechanisms mediating protective immunity against SIV

“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

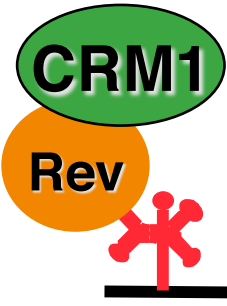
Posttranscriptional Regulation Is Essential for Retrovirus and Retroelement Expression



HIV-1

SRV/D

Intracisternal
A-Particle Retroelements



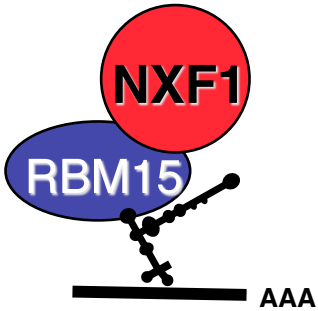
RRE (HIV + lentiviruses)
 Rex/RXRE (HTLV)
 Rec/RcRE (HTDV/HERV)
 Rem/RmRE (MMTV)



CTE (SRV)



CTE (IAP)



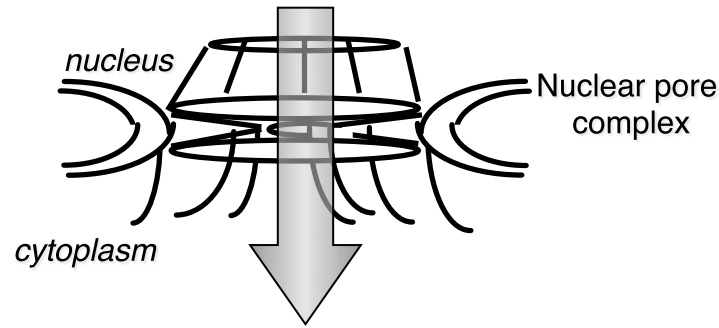
RTE (IAP)

CRM1 nuclear
Export pathway

*NXF nuclear
export pathway*

(Cellular proteins;
 U snRNP;
 Ribosomal subunits)

(spliced cellular
 mRNAs)



EXPORT

Identification of the Cellular Factors Binding to RTE

RTE binding factors:

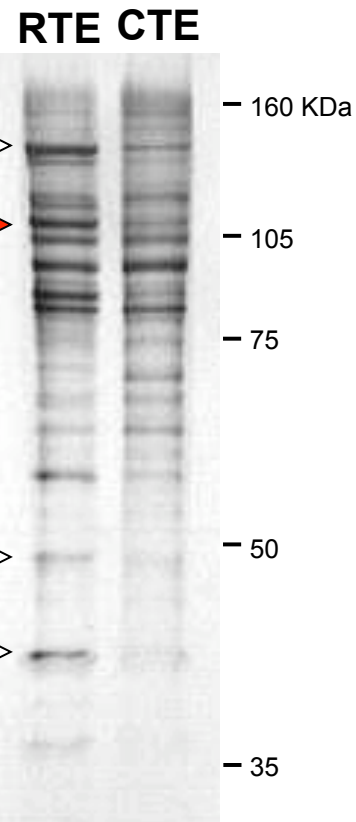
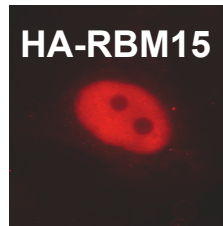
RNA helicase A ⇨

RNA Binding Motif protein 15 (RBM15) ⇨

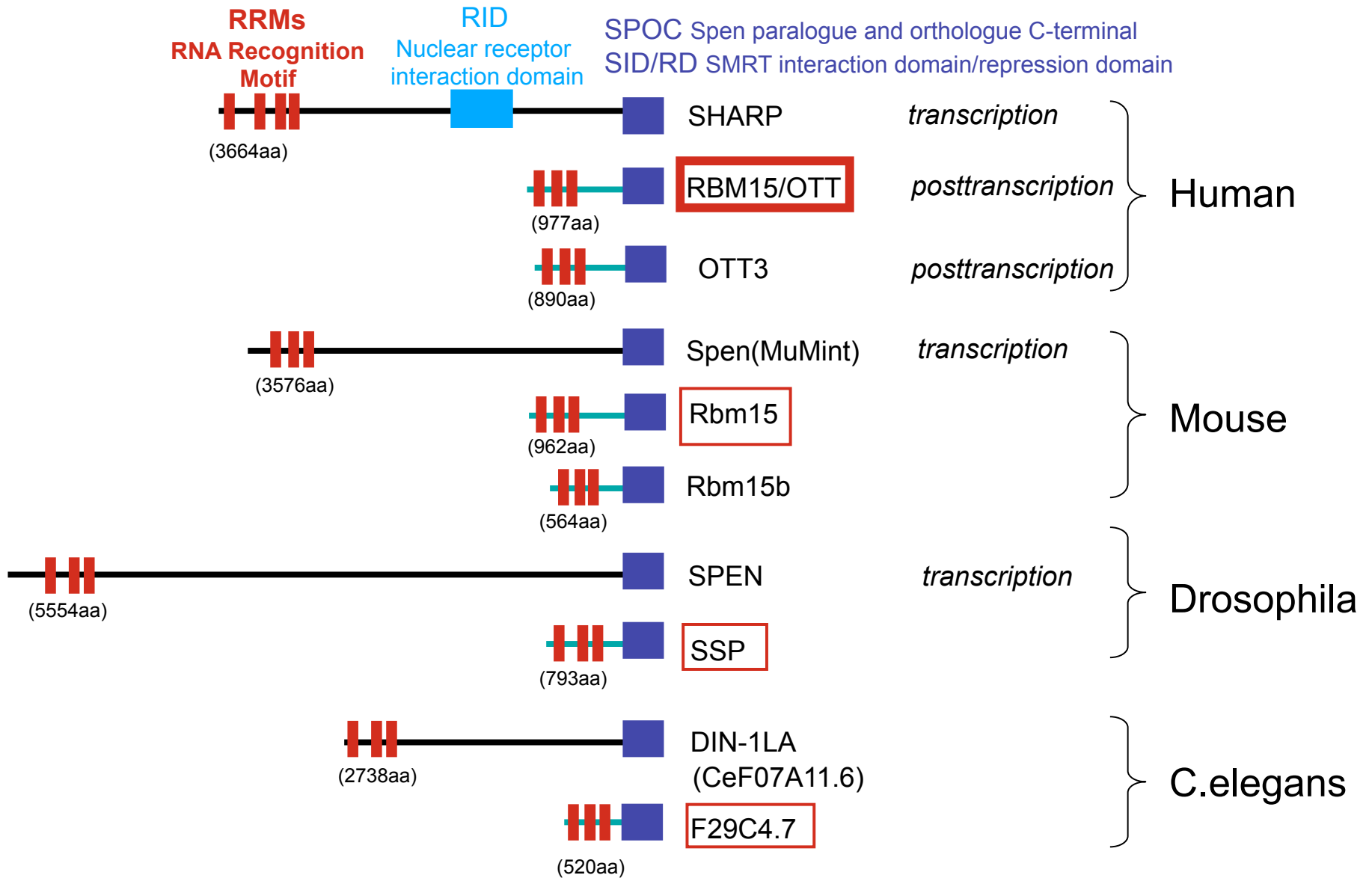
Contains 3 conserved RNA-binding domains (RRM) at N-terminus

Nuclear protein

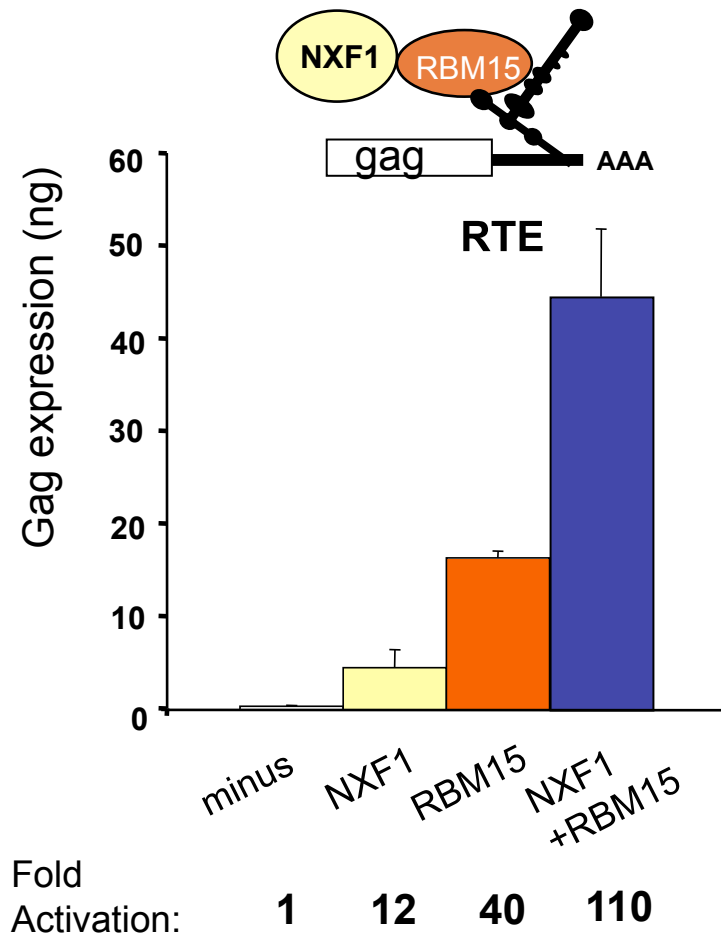
HA-RBM15



RBM15 belongs to Spen (Split end) Family of Proteins

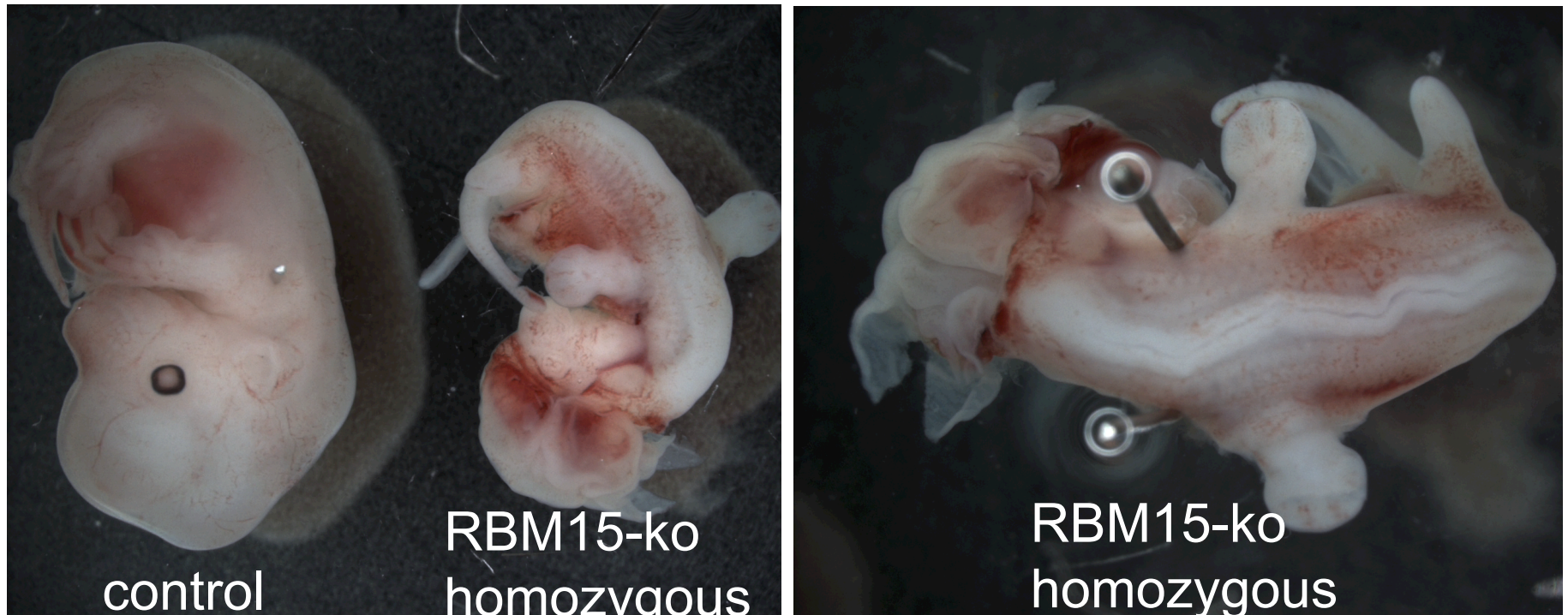


RBM15 Tethers RTE-RNA to the NXF1 Export Pathway



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

RBM15 is Essential for Mouse Development



Day 11.5

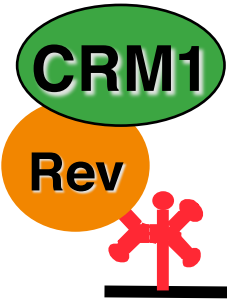
Preliminary study indicates a role of RBM15 in the expression of a subset of cellular mRNAs

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

HIV-1

SRV/D

Intracisternal
A-Particle Retroelements



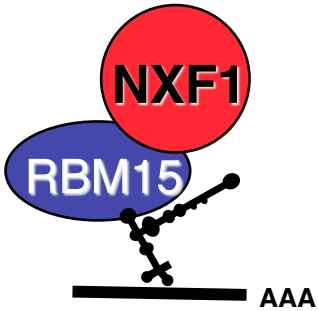
RRE (HIV + lentiviruses)
 Rex/RXRE (HTLV)
 Rec/RcRE (HTDV/HERV)
 Rem/RmRE (MMTV)



CTE (SRV)



CTE (IAP)



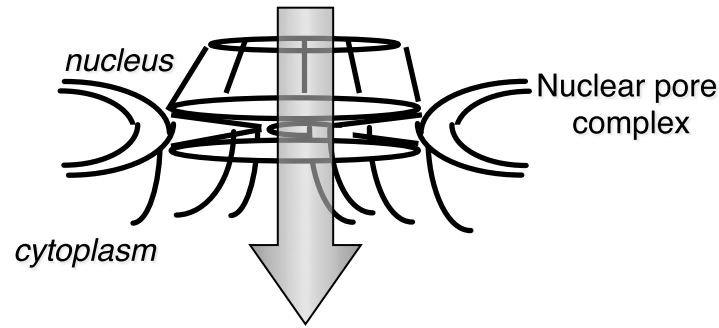
RTE (IAP)

CRM1 nuclear
 Export pathway

*NXF nuclear
 export pathway*

(Cellular proteins;
 U snRNP;
 Ribosomal subunits)

(spliced cellular
 mRNAs)

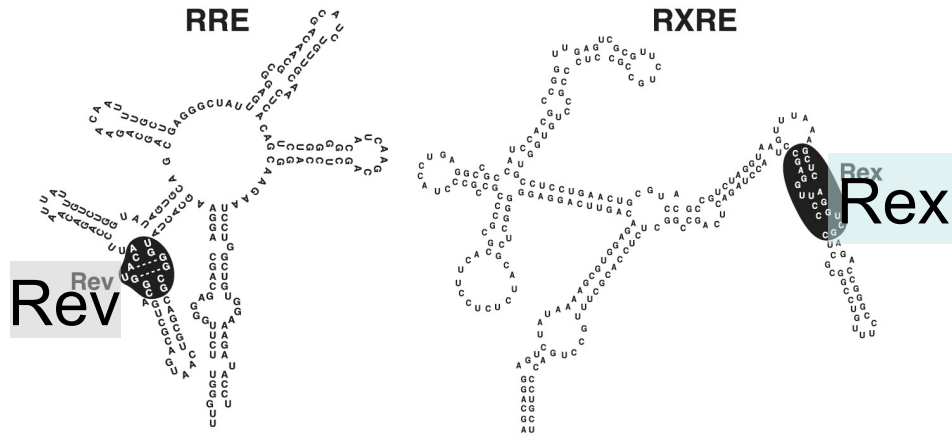


EXPORT

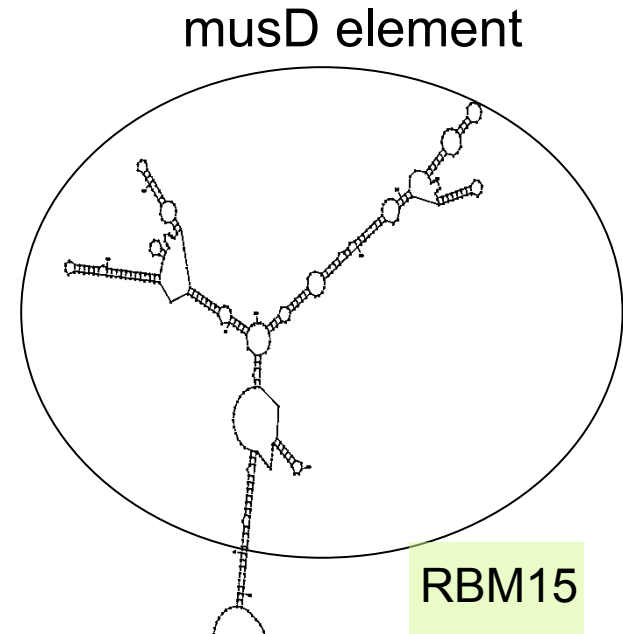
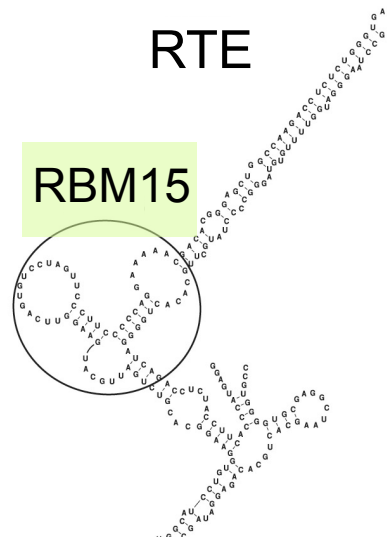
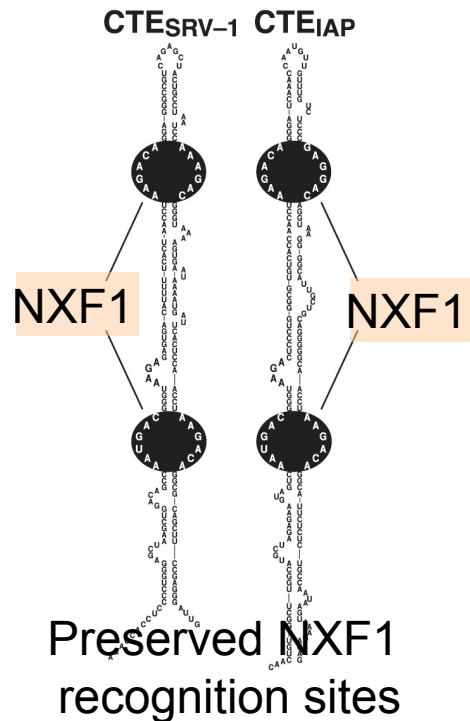
Retroviruses and Retroelements Utilize Distinct RNA Export Mechanisms

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



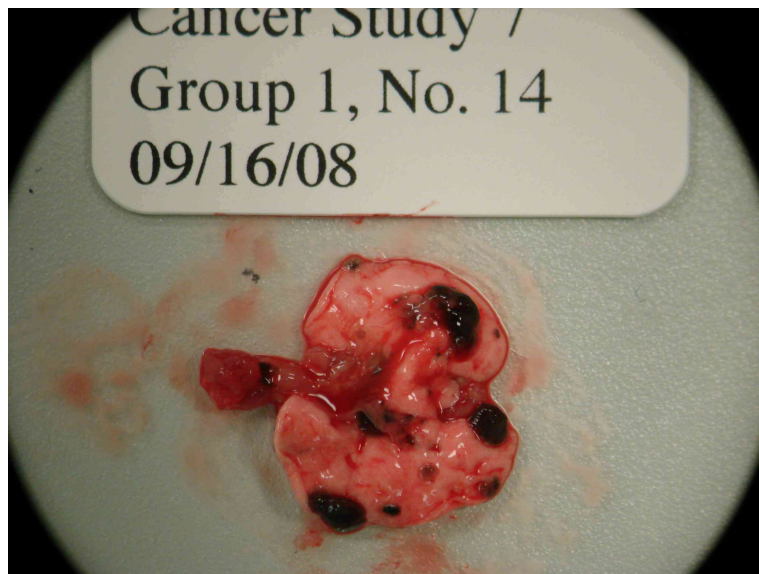
- Different sequence
- Different RNA structure
- Different binding factors



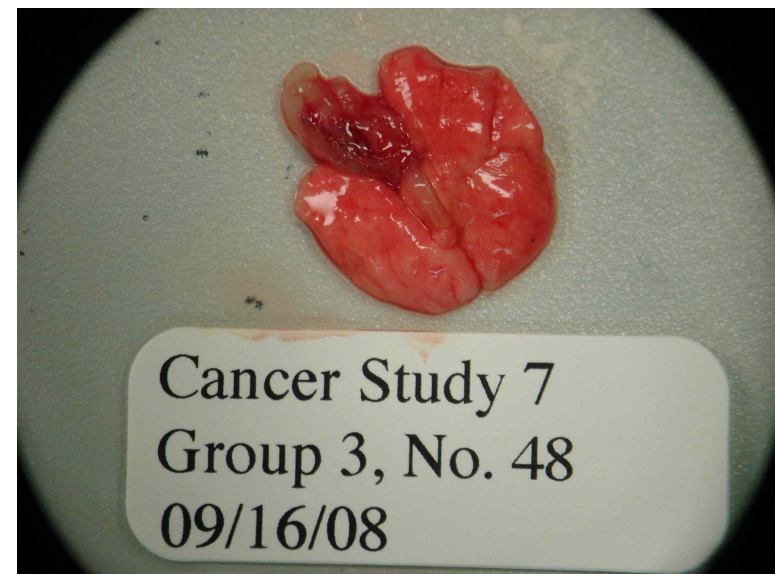
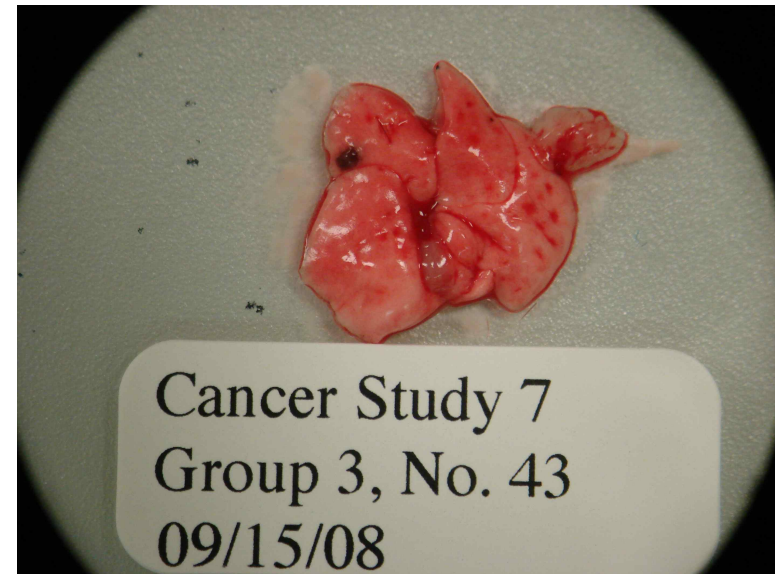
RBM15 binds to RTE and musD element
 • suggests conformational recognition site(s)

Study Cancer 7: LUNG TUMOR MODEL

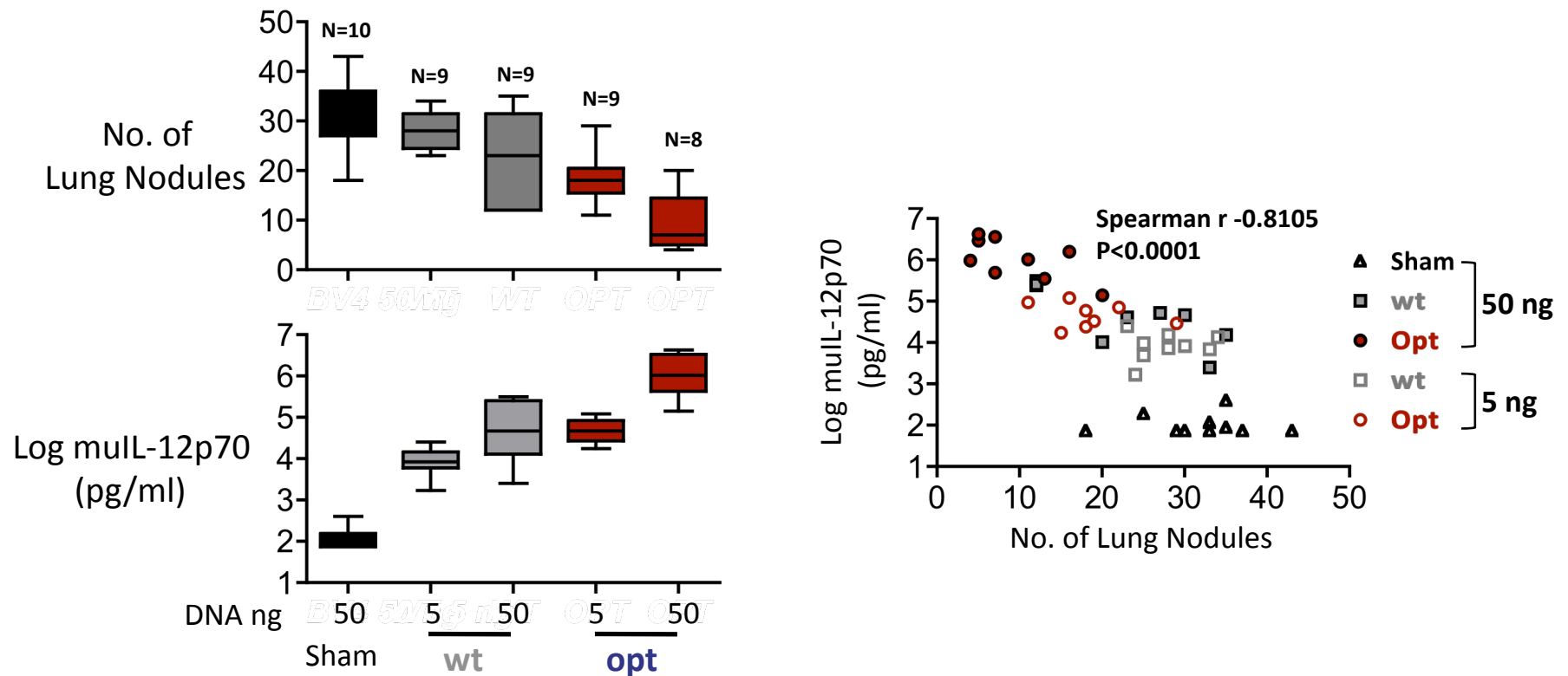
Control



IL-12



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



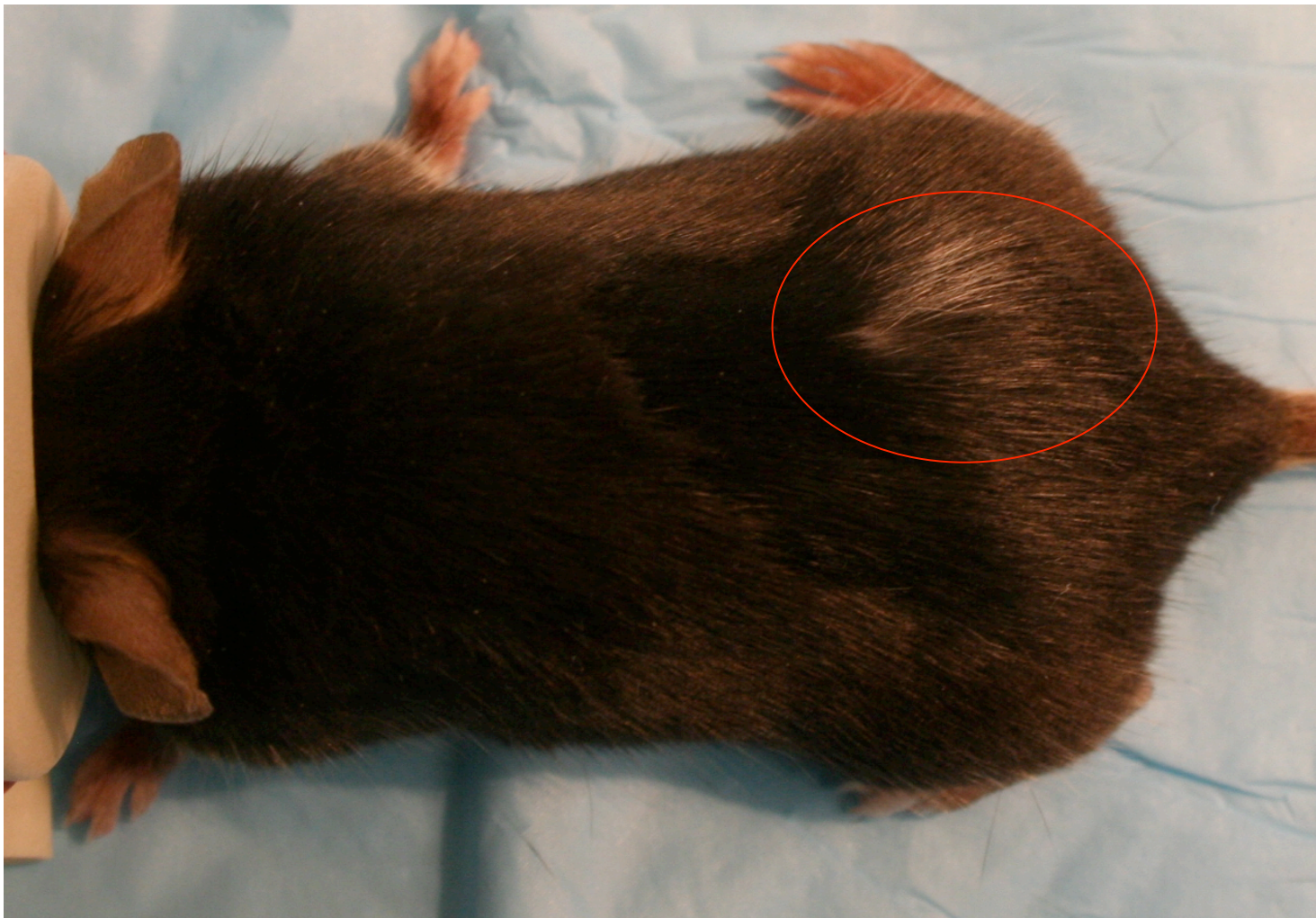
Lung tumor model:

Day 0: intravenous injection of 3×10^5 B16 Melanoma cells

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

Day 22: Sacrifice mice and count lung and liver nodules

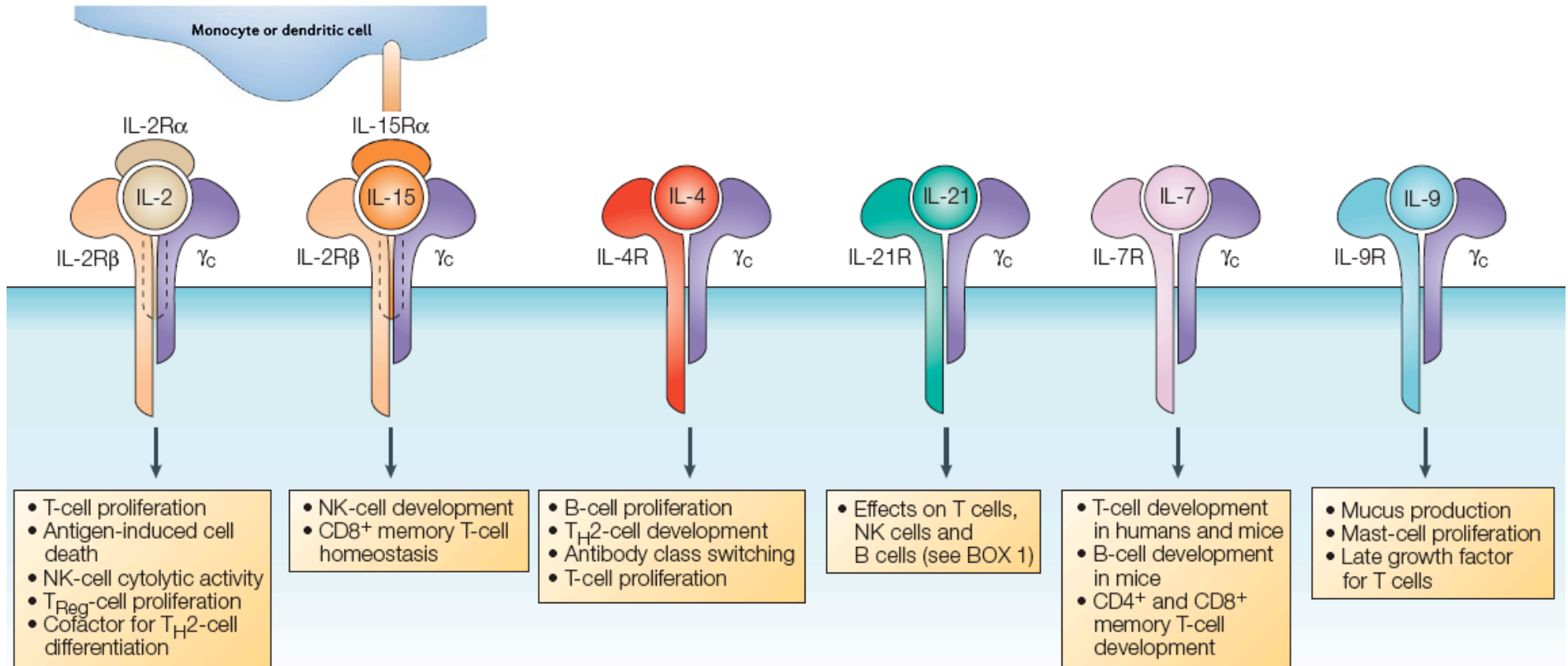
Vitiligo In A Mouse Eliminating Large Vascularized B16
Melanoma After IL-12 DNA IT Injection:
Suggests Immune Activation Participates In Tumor Remission



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

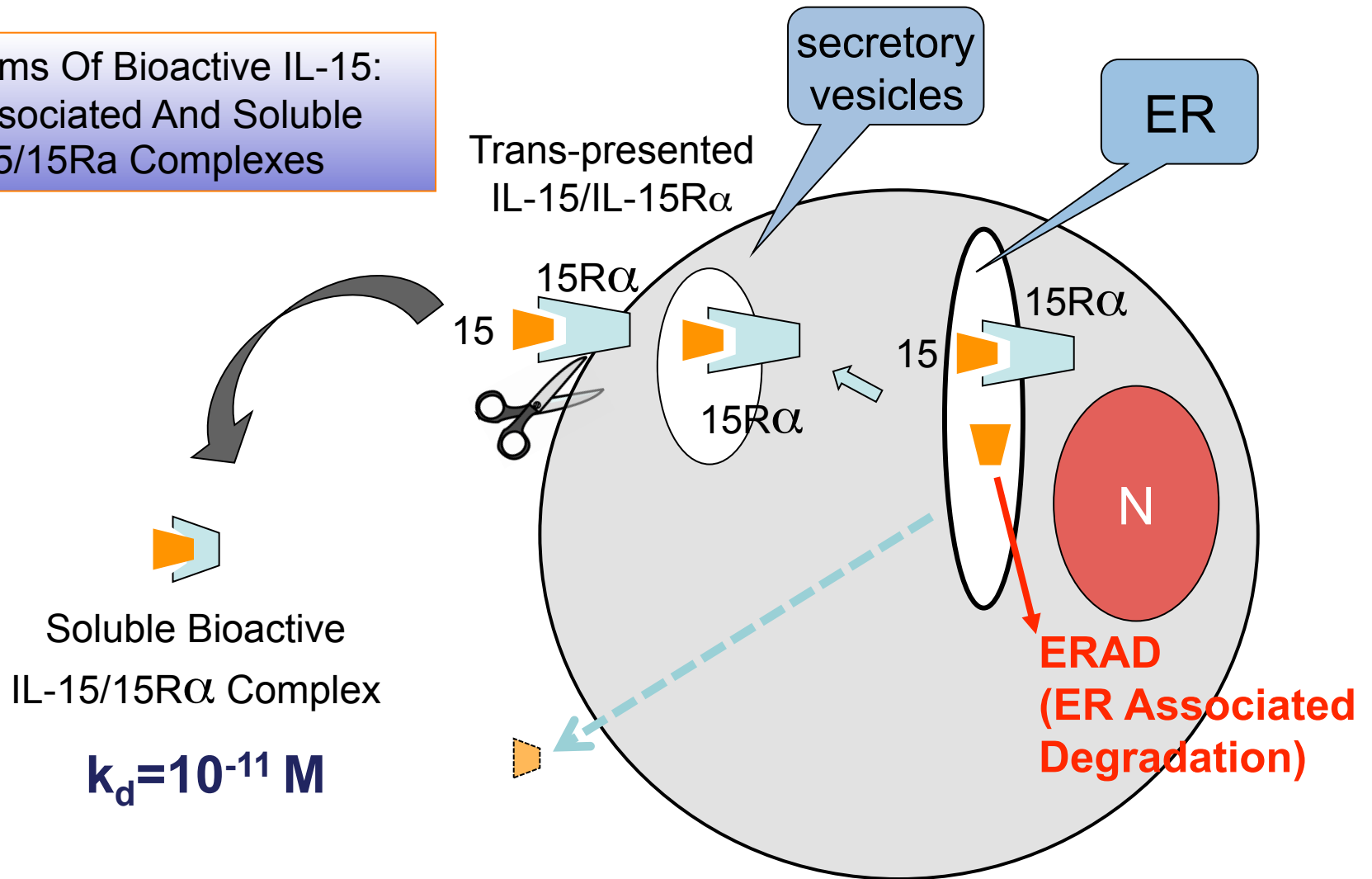
George N Pavlakis
NCI

Common γ_c Chain Cytokines



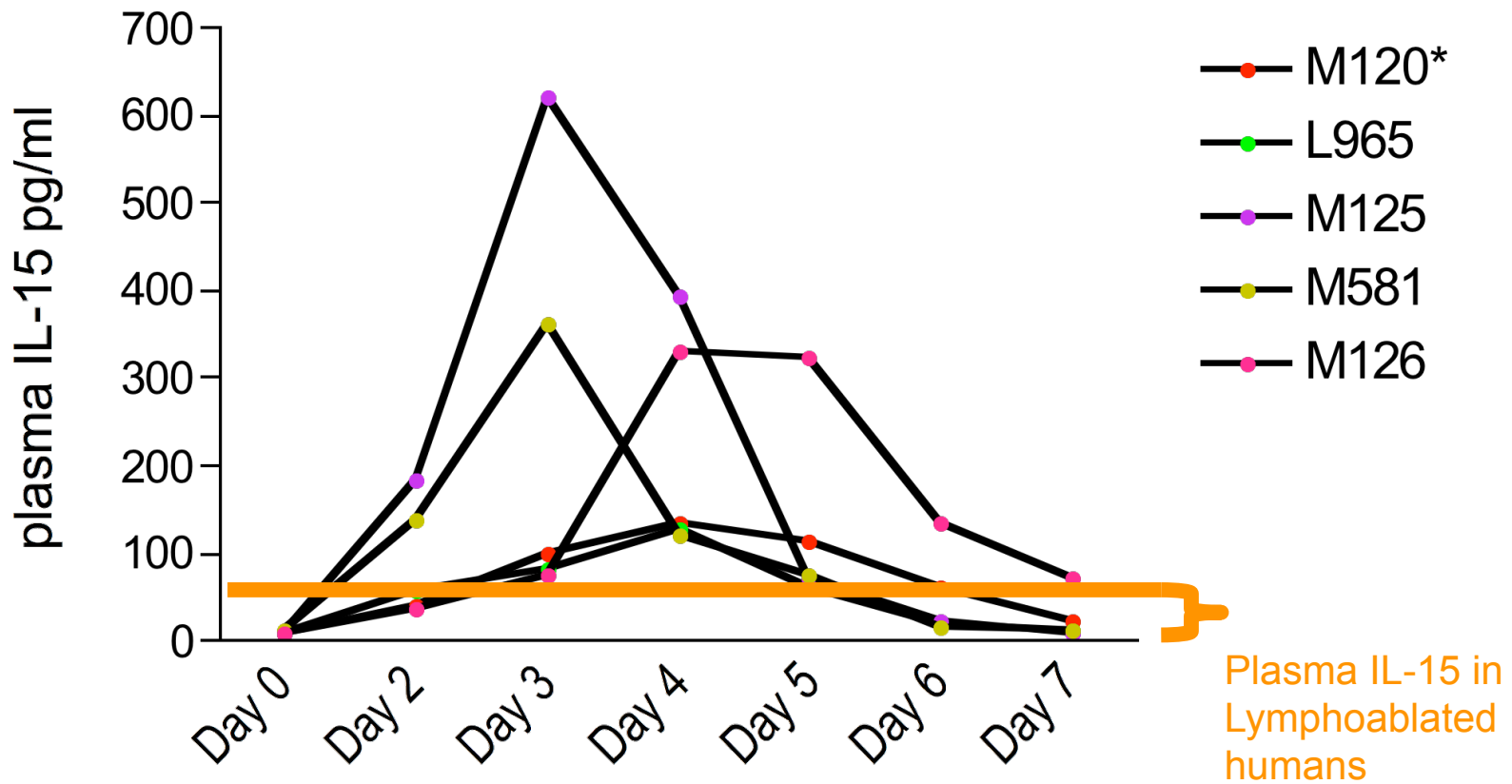
Co-production Leads To Mutual Stabilization of IL-15 and IL-15R α

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





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

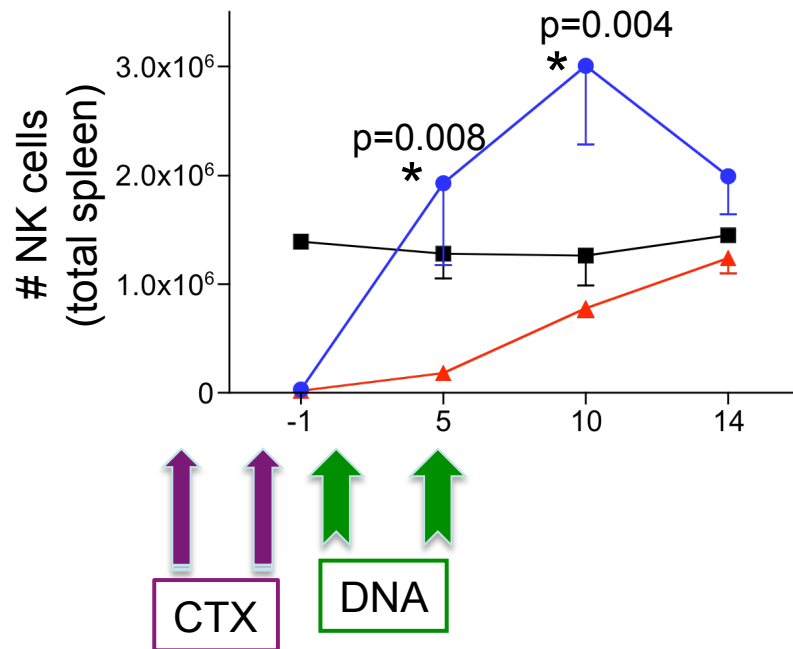


Recovery of NK cells

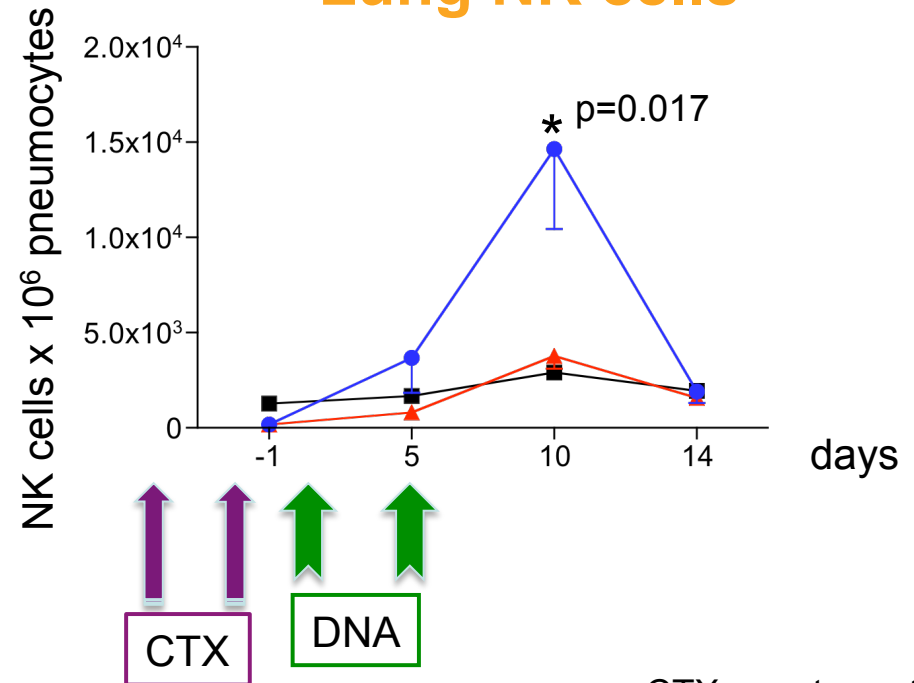


■ Control
 ▲ CTX+empty vector
 ● CTX+IL-15

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

