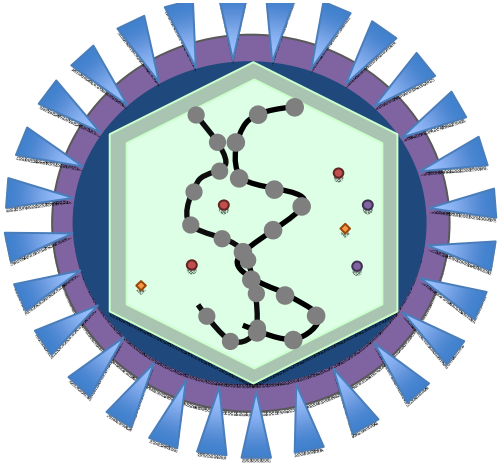
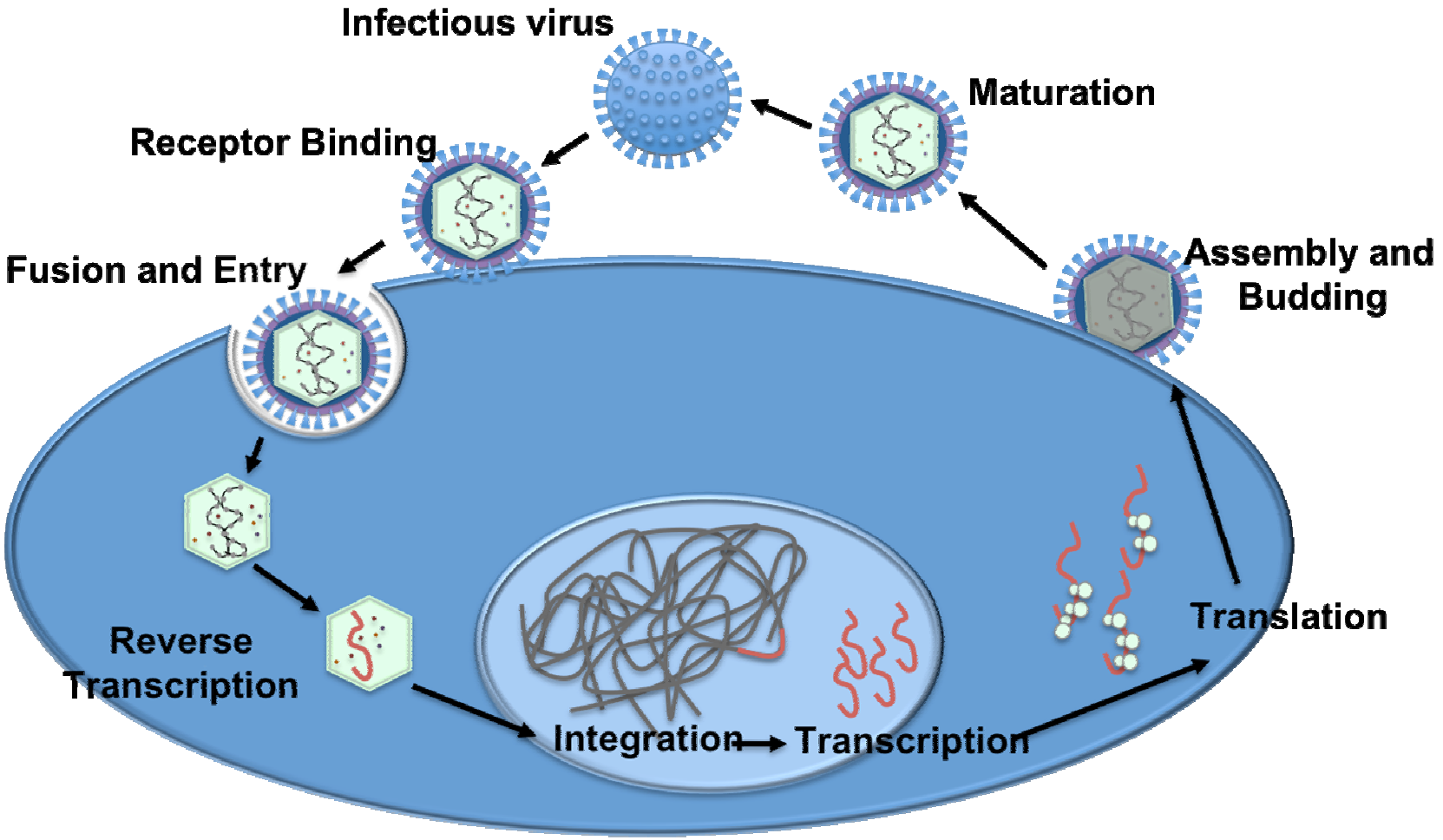


Retroviral RNA Processing and stability

Karen Beemon

Johns Hopkins University

Retroviruses hijack host cell gene expression machinery to generate progeny virions

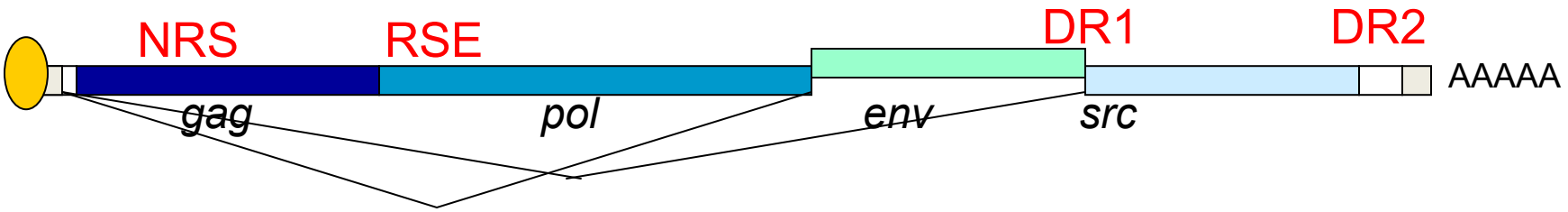


Simple retroviruses use cis-acting RNA sequences to express unspliced RNA

1) Stability Element (RSE) (*pol*): Stabilize RNA with long 3'UTR

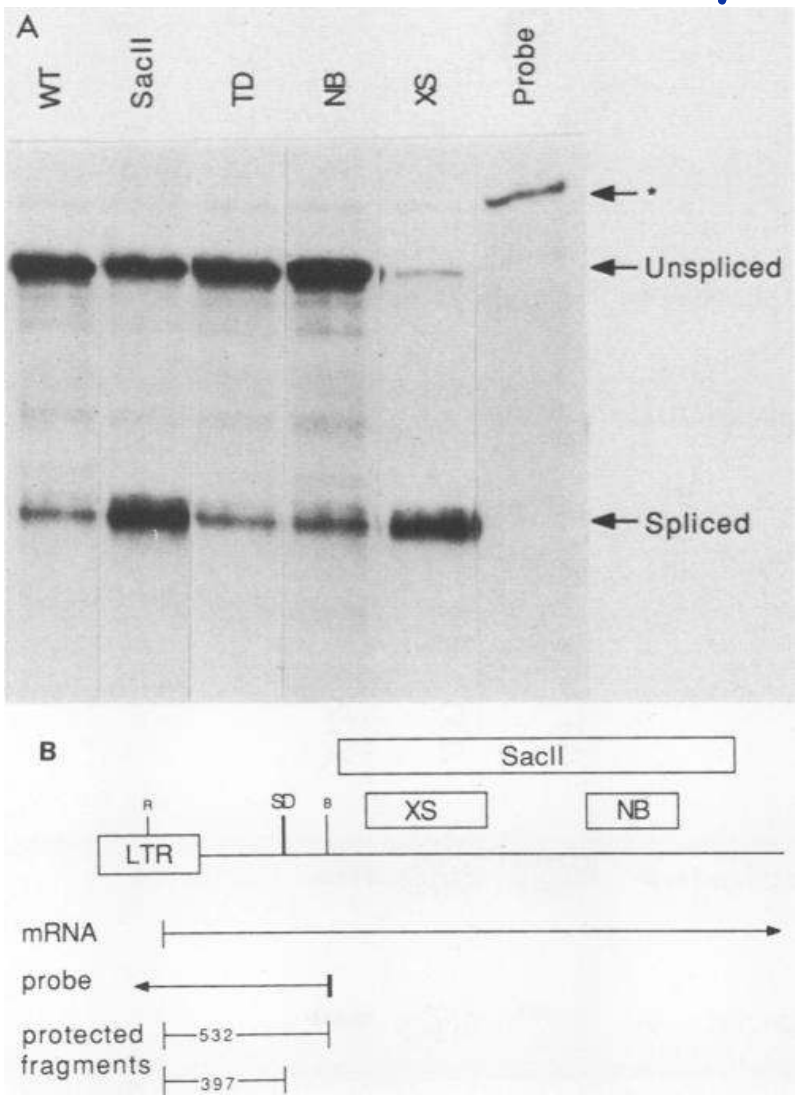
2) Negative Regulator of Splicing (NRS) (*gag*): suppresses splicing, promotes pA (pseudo-5' SS:binds U1/U11 & SRs), promotes rapid lymphomas

3) Direct Repeat (DR) (3'UTR): Exports unspliced RNA (virion assembly) Needs Tap and Dbp5.



Rous sarcoma virus RNA

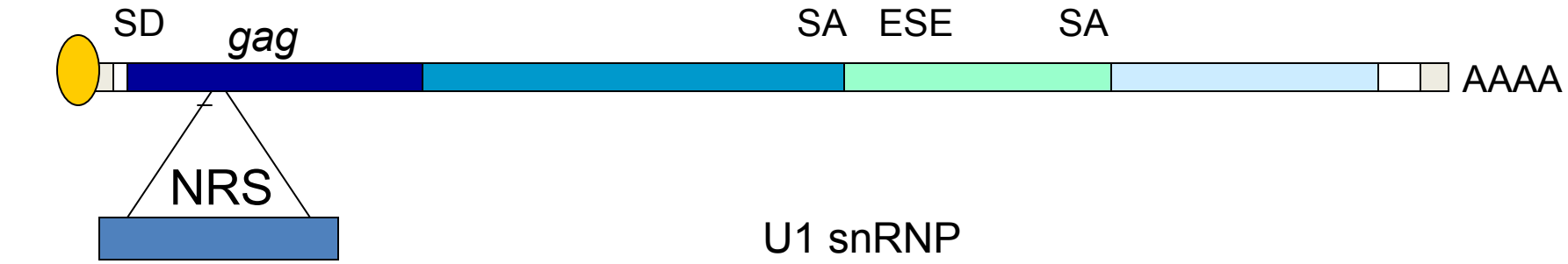
Deletions in gag affect RSV RNA splicing and stability



Arrigo and Beemon
MCB 1988

SPLICING: How is the correct ratio of spliced/ unspliced viral RNAs maintained?

- 5’ splice site efficient, 3’ splice sites inefficient (RSV & HIV)
- Exonic splicing enhancers and silencers
- Negative regulator of splicing (NRS) sequence in *gag*
  - 5’ splice site decoy, 500 nts downstream of 5’ splice site
  - Also promotes pA at 3’ LTR



U1 snRNP

uG/GUuuGUAUCCUUC

NRS

U11 snRNP

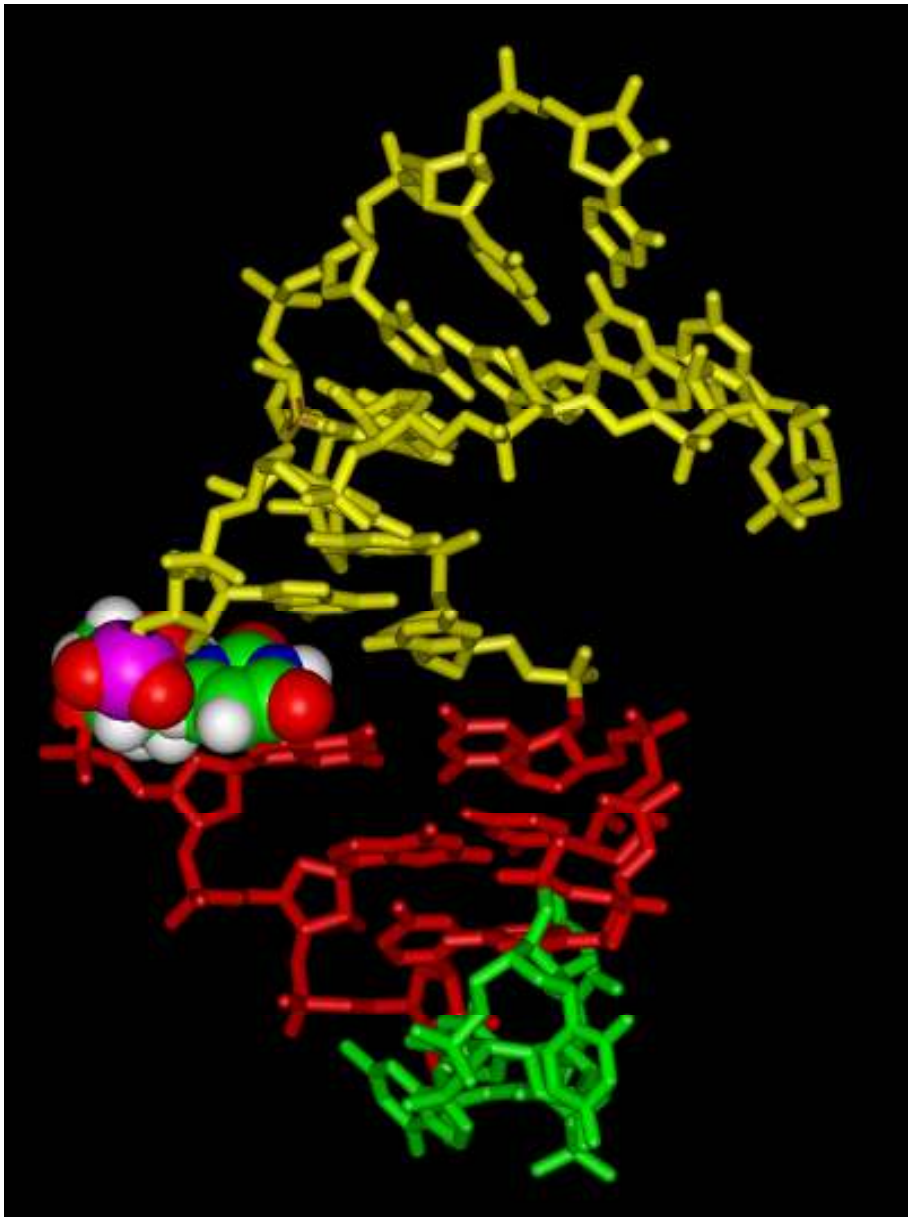
AA: spl.

uG/GUAAGUAUCCUUC

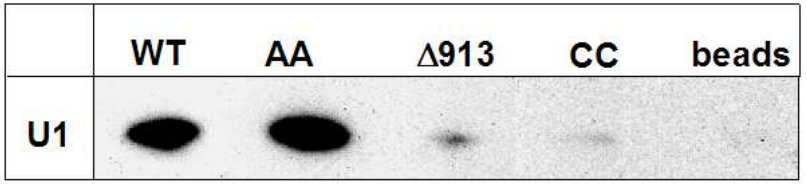
CC: 0

uG/GUccGUAUCCUUC

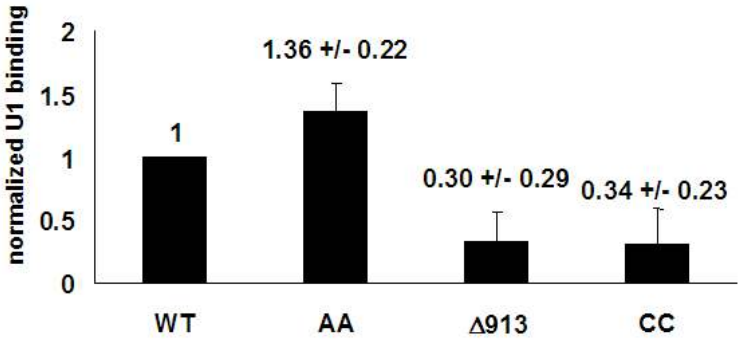
Bulged U in NRS 5’ helix promotes U1 snRNP binding



A



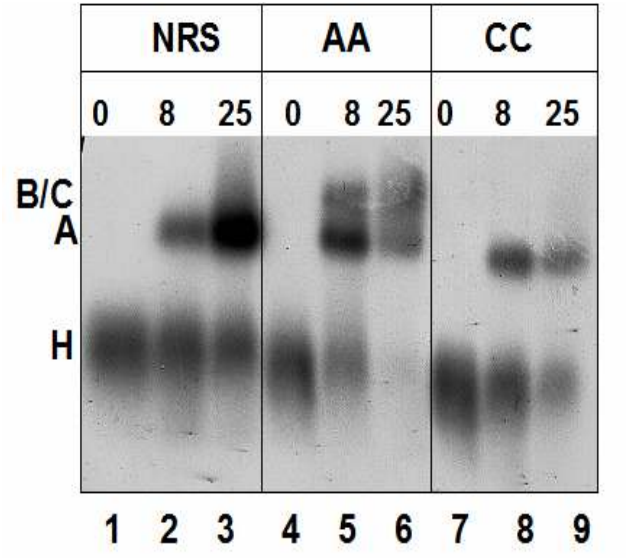
B

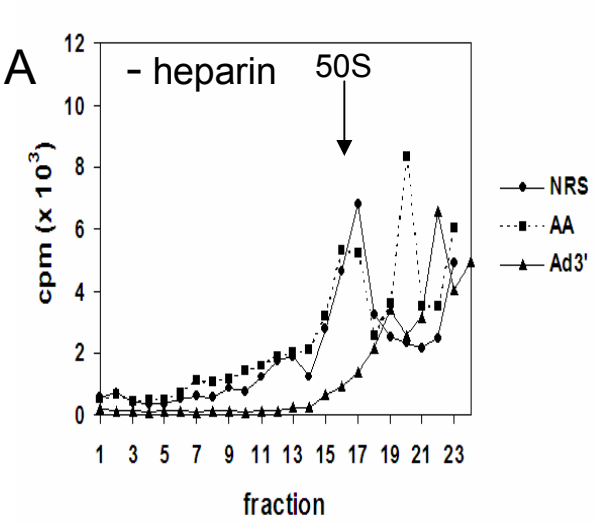


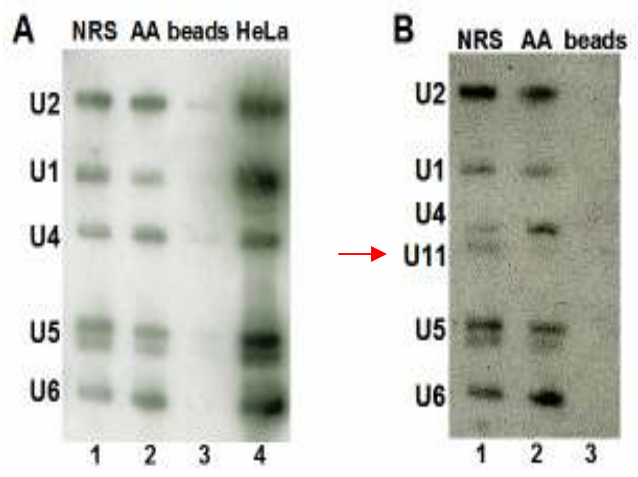
uG/GUuuGU

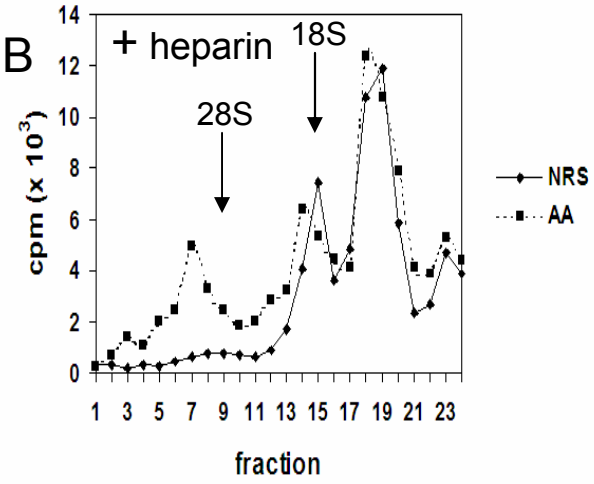
Cabello-Villegas et al. RNA 2004
(Yun-Xing Wang, NCI)

NRS-Ad3' RNA makes aberrant 50S spliceosomal complex



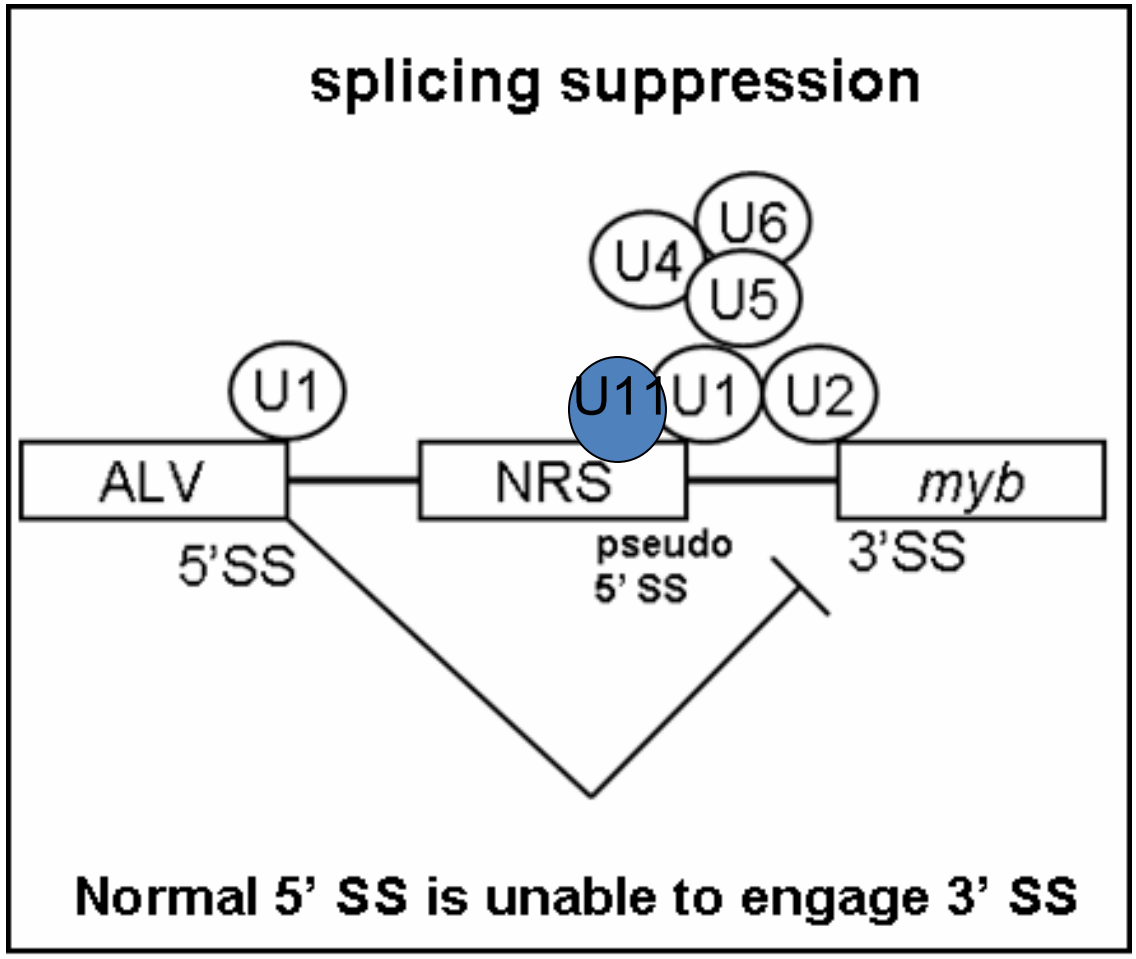




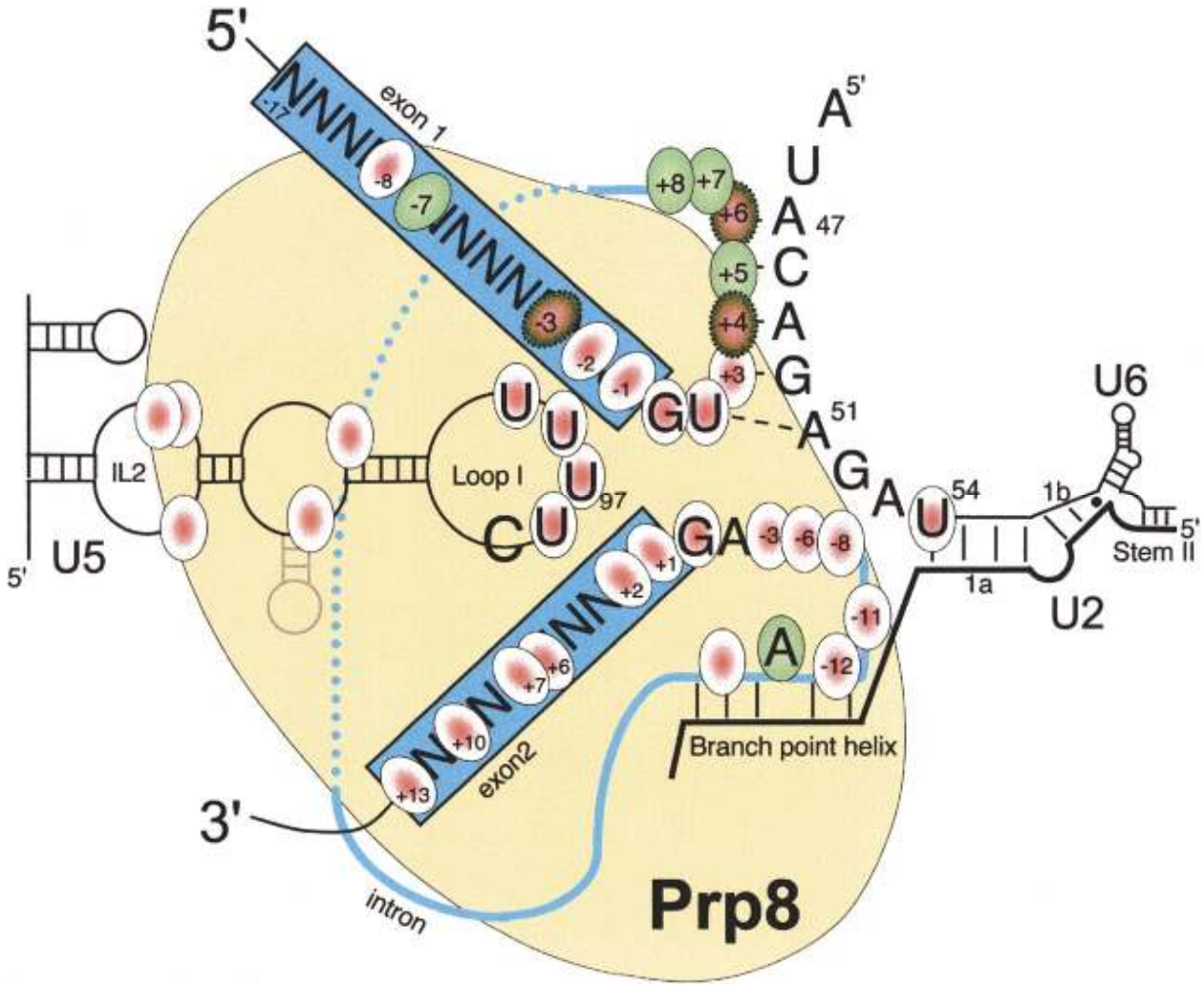


Giles & Beemon, MCB 2005

NRS sequesters 3'SS in aberrant complex

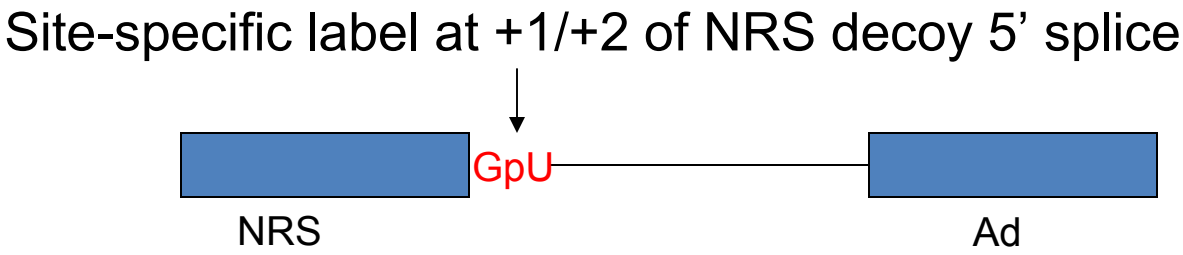


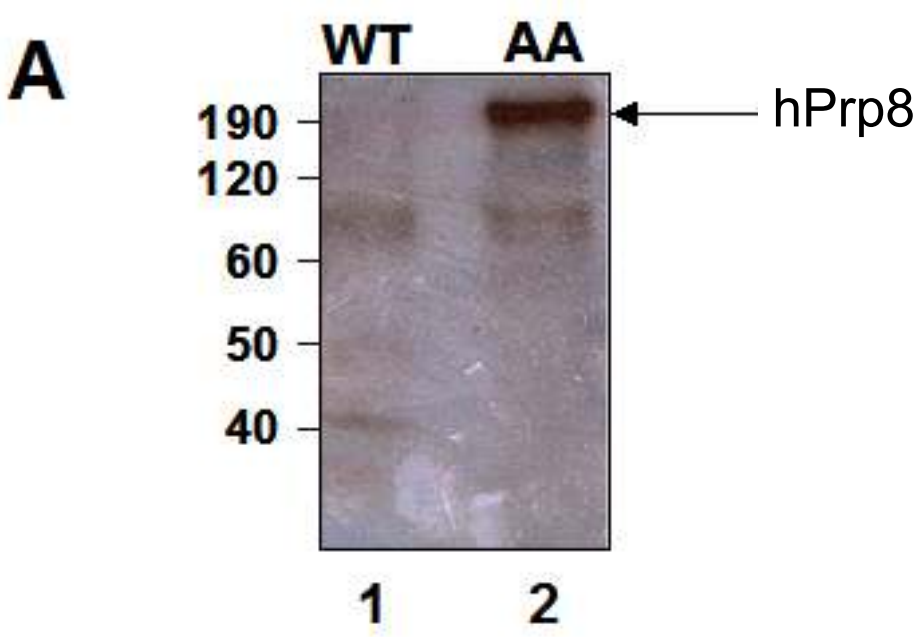
U5 Prp8 is critical to spliceosome function, cross-links to 5'SS



GRAINGER et al. RNA 2005; 11: 533-557

U5 Prp8 does not X-link to the NRS 5' splice site





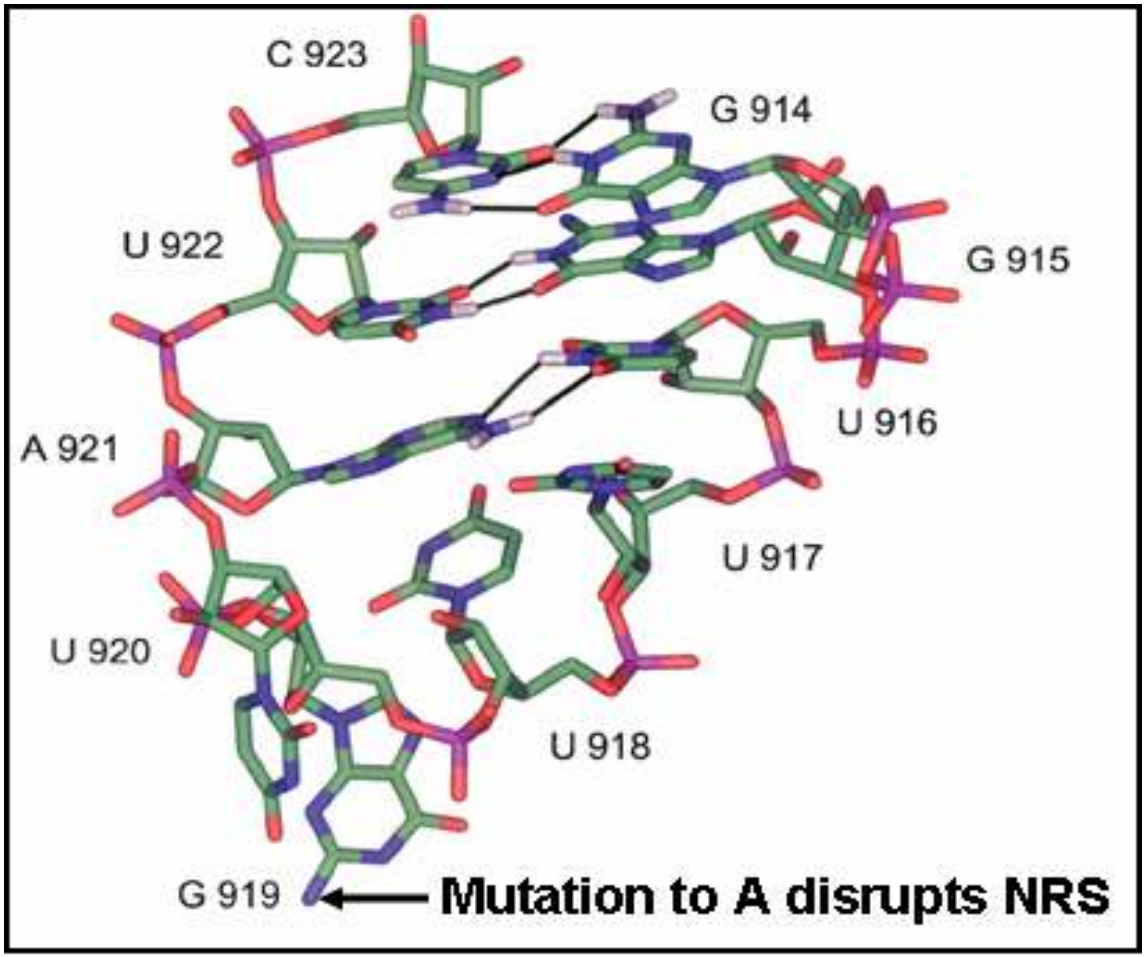
Giles & Beemon, MCB 2005

1. The NRS is a 5’ splice site decoy that inhibits splicing and promotes polyadenylation.

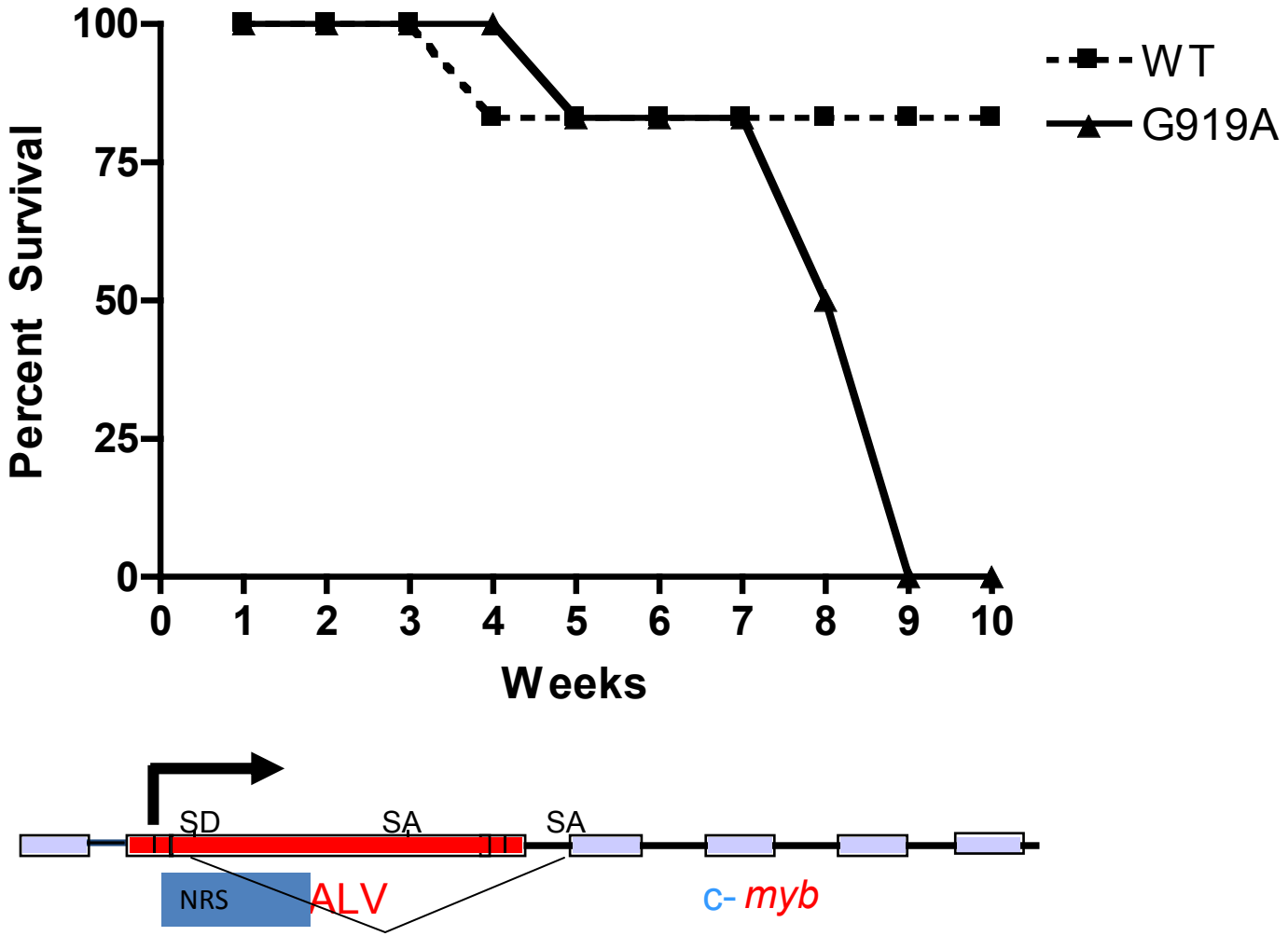
2. U5 Prp8 is misplaced.

How do NRS mutant ALVs act in vivo?

Mutation at G919A (+5) converts splicing suppressor to 5′ SS



ALV with NRS point mutation induces rapid-onset lymphomas

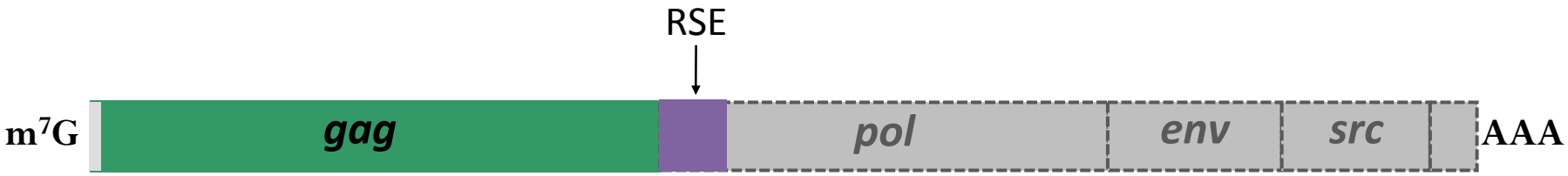


Polony et al. 2003 JVI

Retroviral Stability Element (RSE)

Prevents Nonsense-Mediated mRNA Decay (NMD) of viral RNA with long 3′ UTR

The Rous sarcoma virus unspliced RNA must evade host translation quality control



Long mRNA (>9 kb)

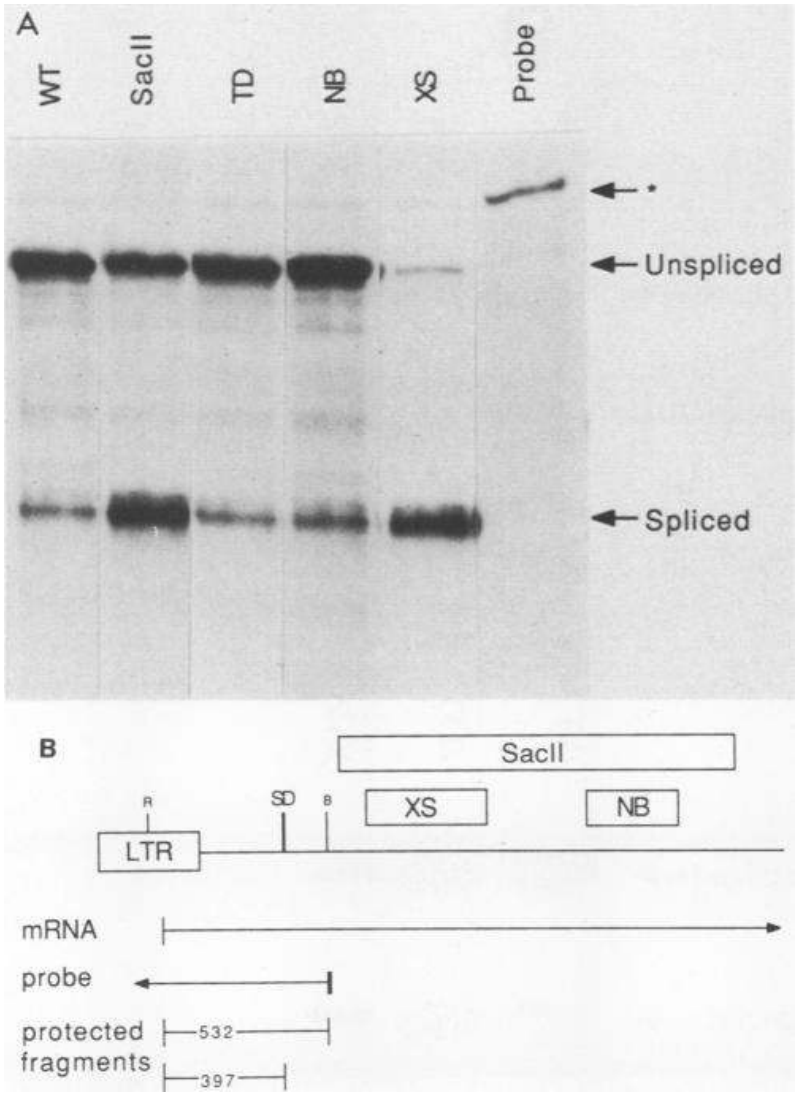
Unspliced mRNA

Polycistronic

Long 3' UTR after the first open reading frame

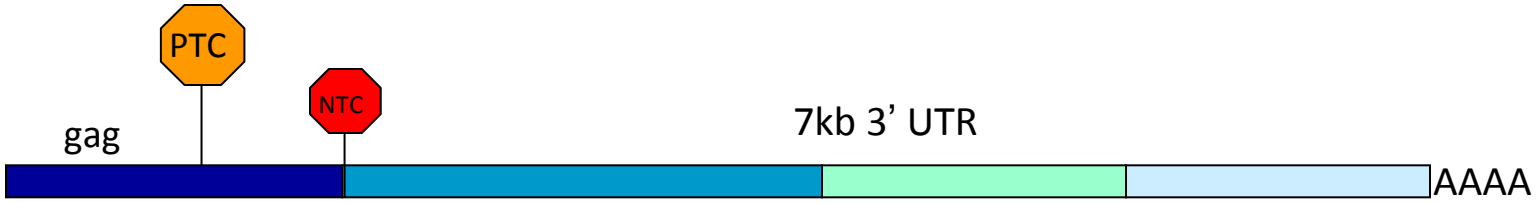
Long half-life (9-20 hrs)

Deletions in gag affect RSV RNA splicing and stability

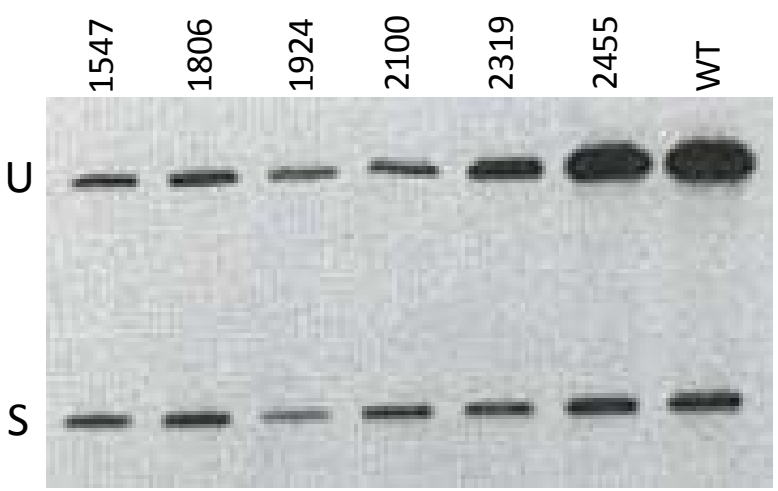


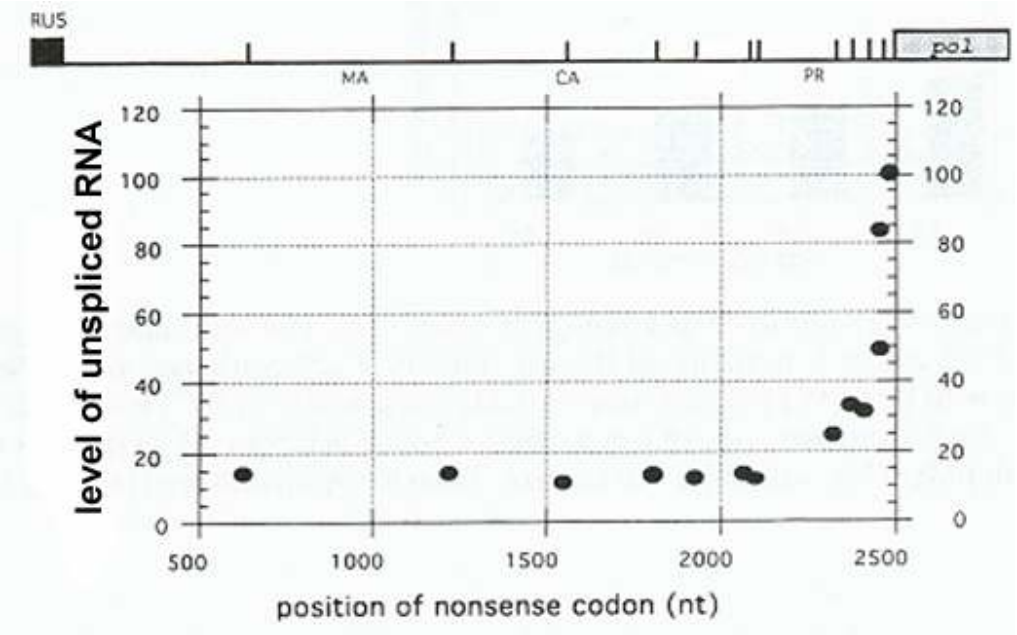
Arrigo and Beemon
MCB 1988

RNA termination codon in *gag* leads to instability of unspliced RNA



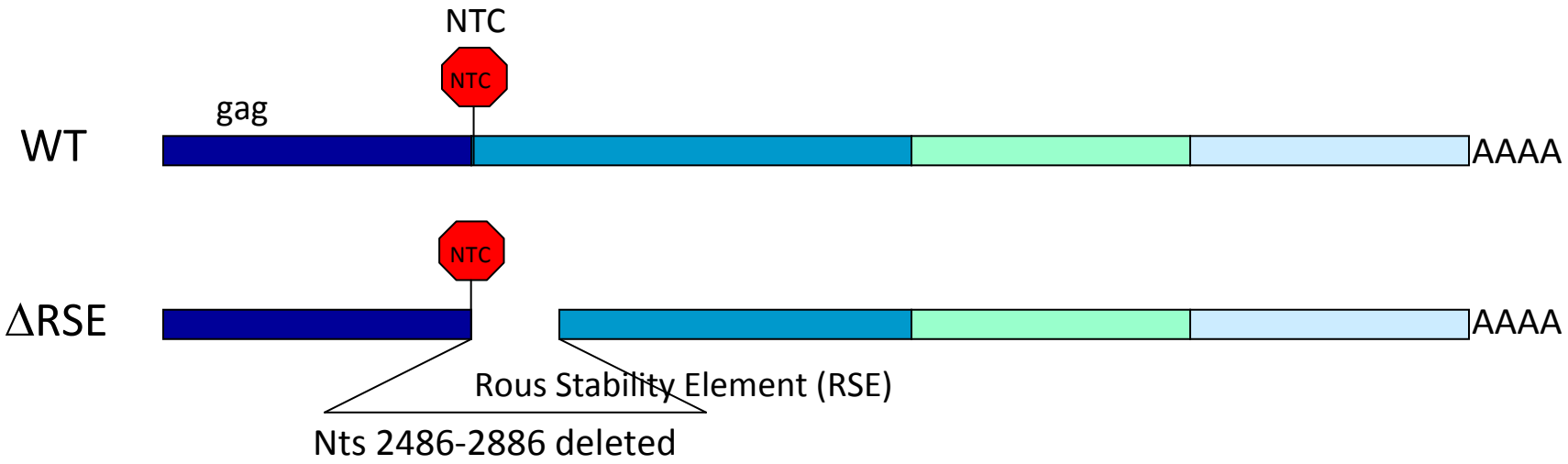
Nonsense codon at nt

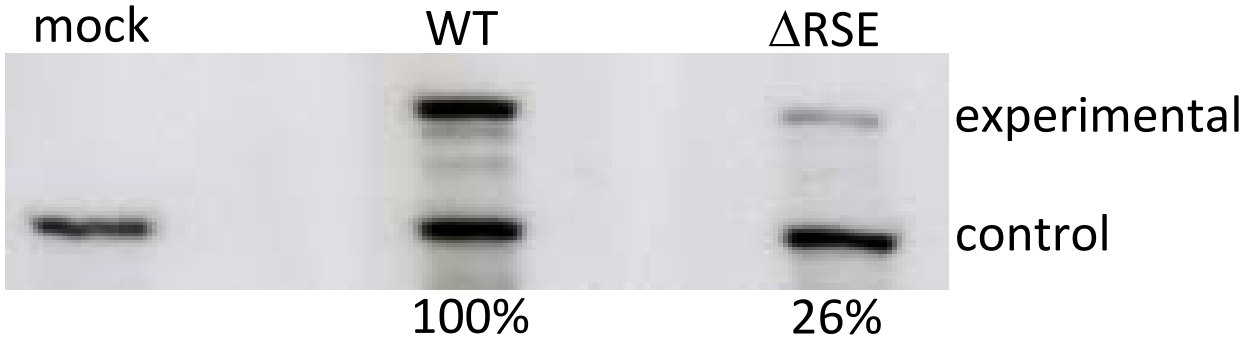




Barker and Beemon, MCB 1994

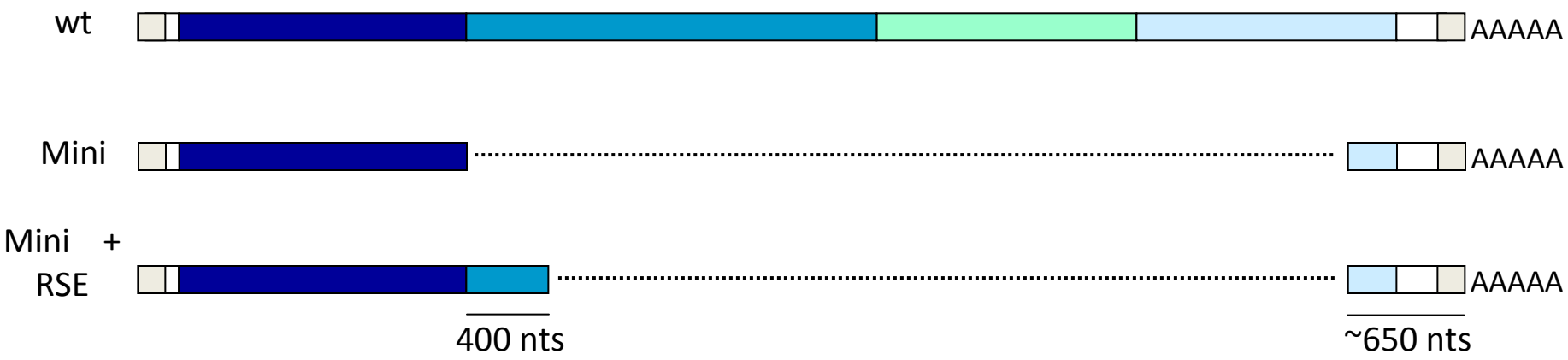
A deletion downstream of *gag* NTC also destabilizes RNA

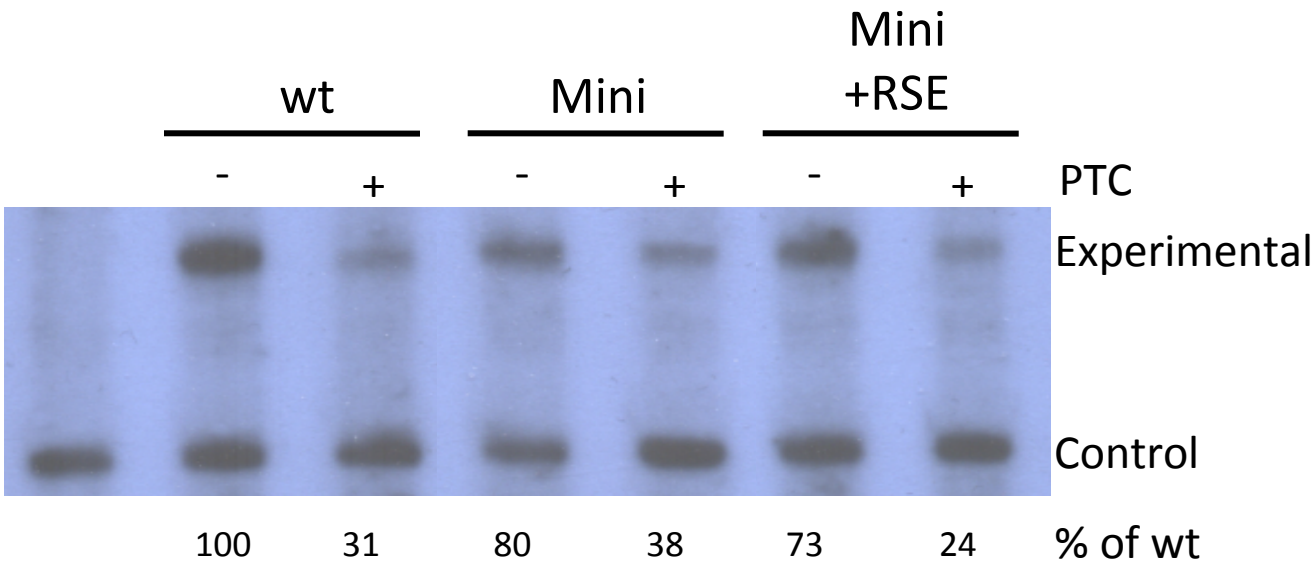




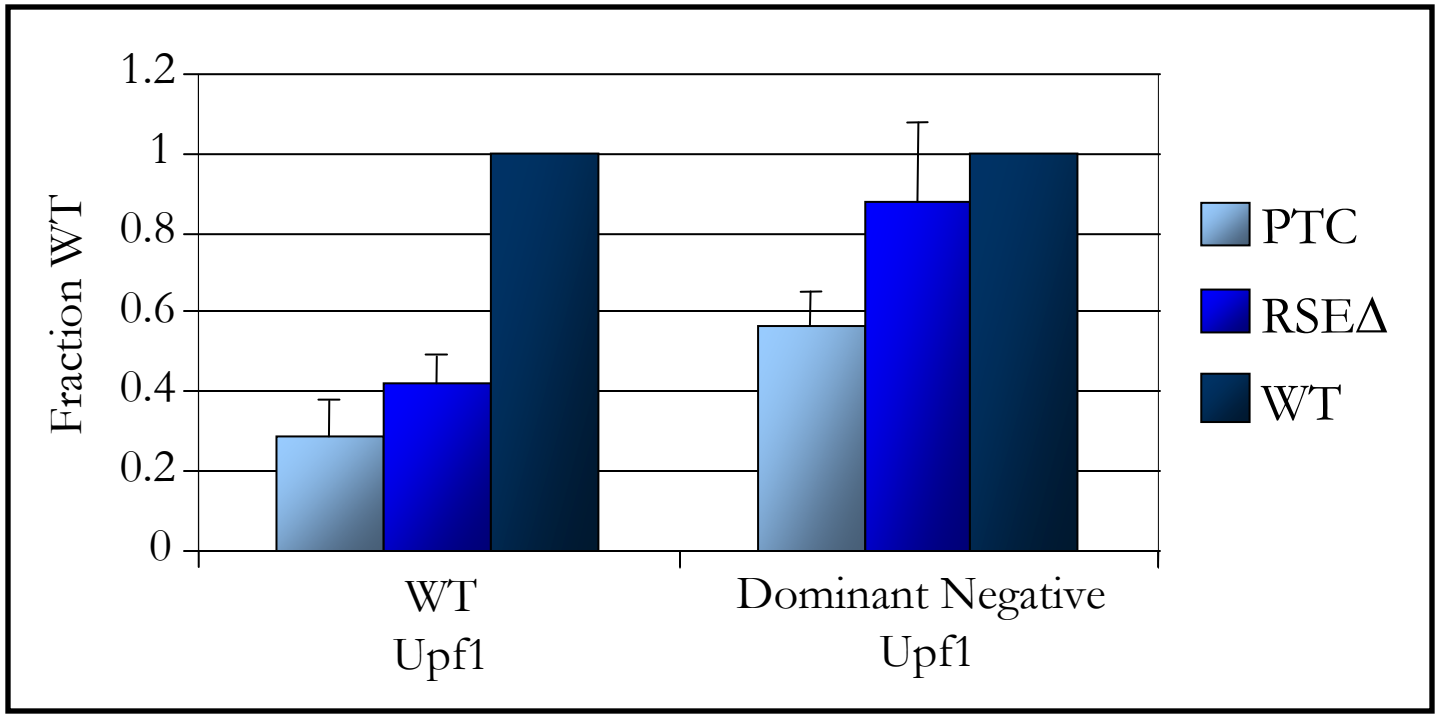
Weil and Beemon, RNA 2006

Stability element is dispensable when termination occurs near the poly(A) tail





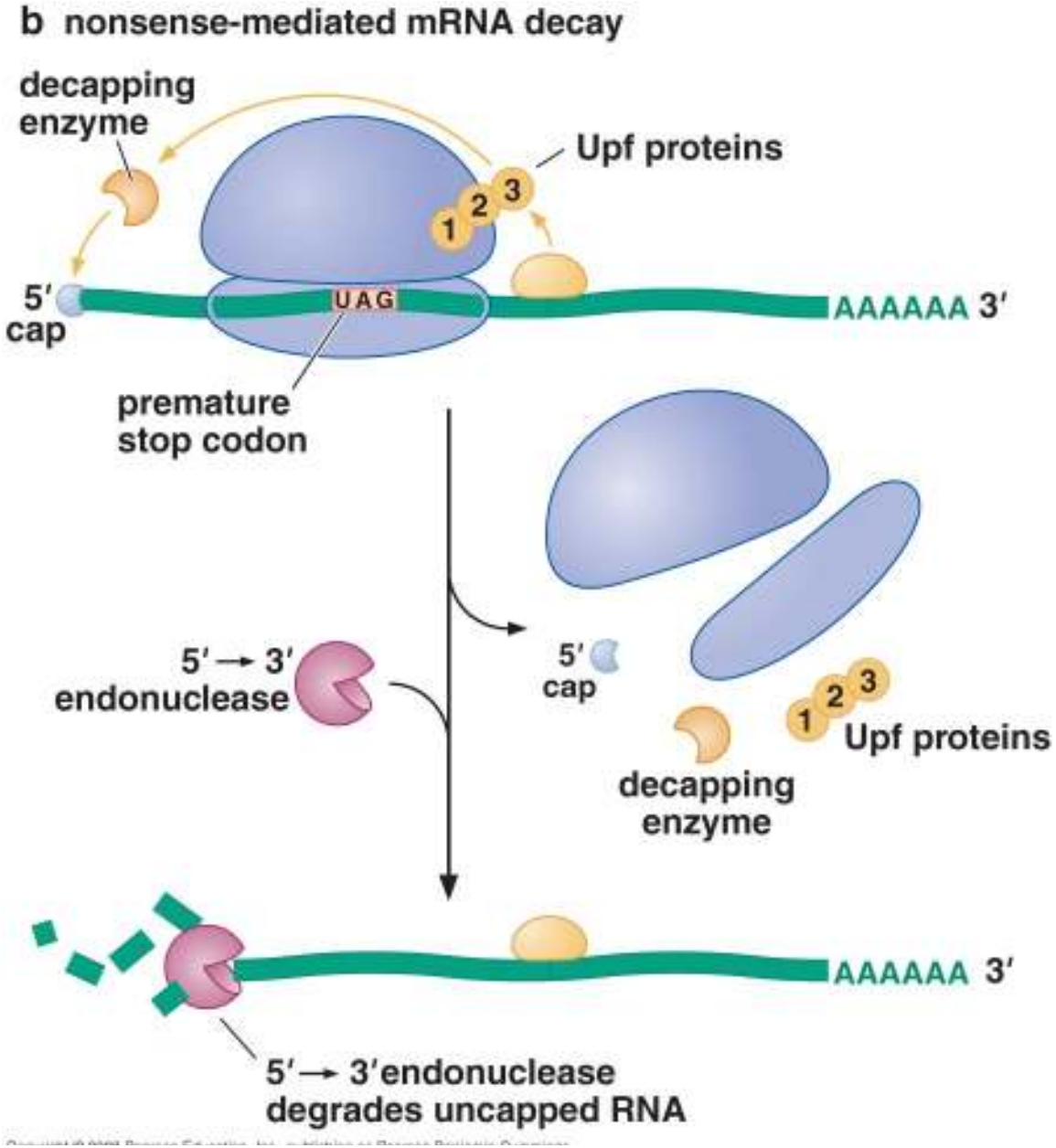
Dominant negative Upf1 stabilizes RNA lacking the RSE



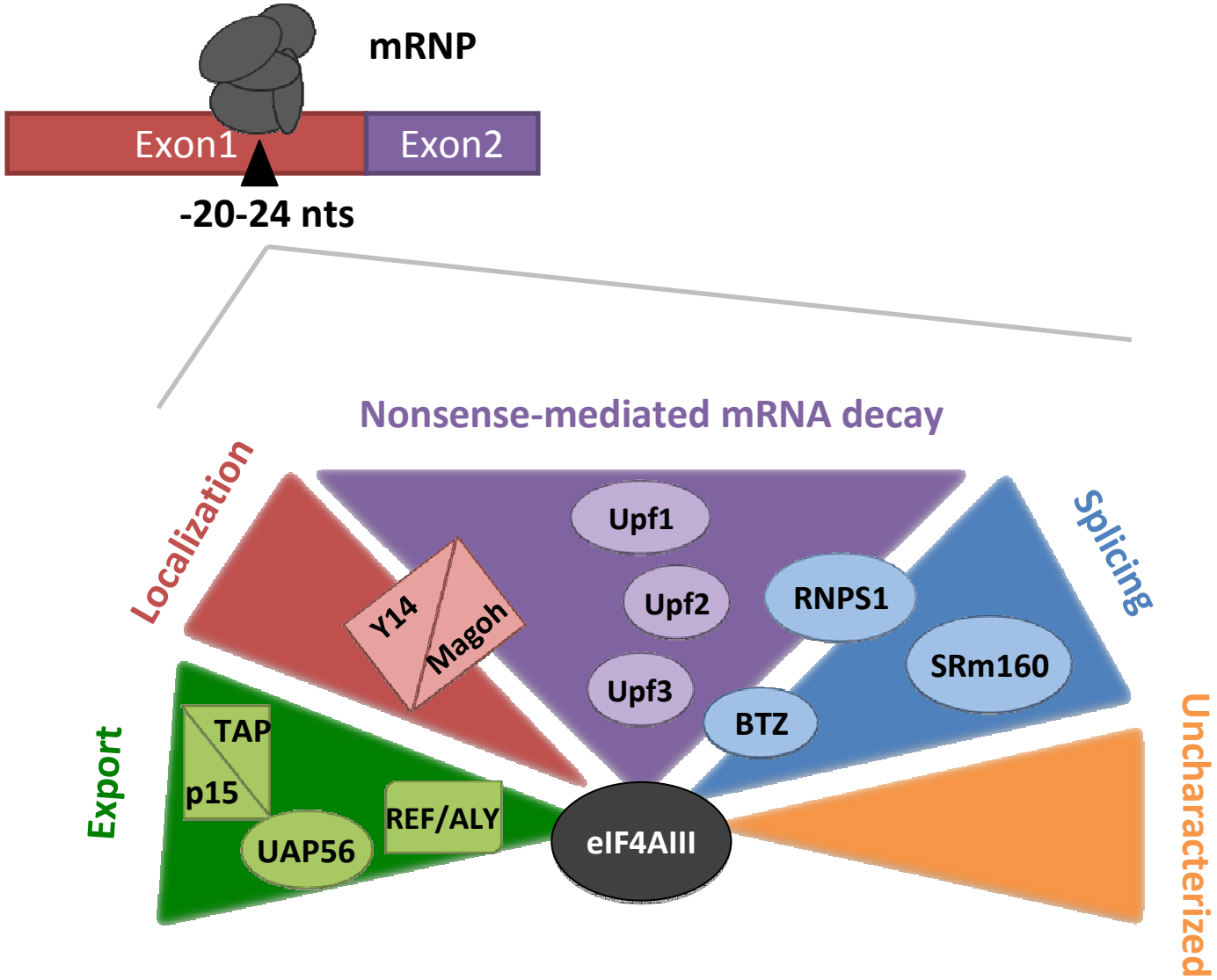
Implicates nonsense-mediated mRNA decay

Weil and Beemon, RNA 2006

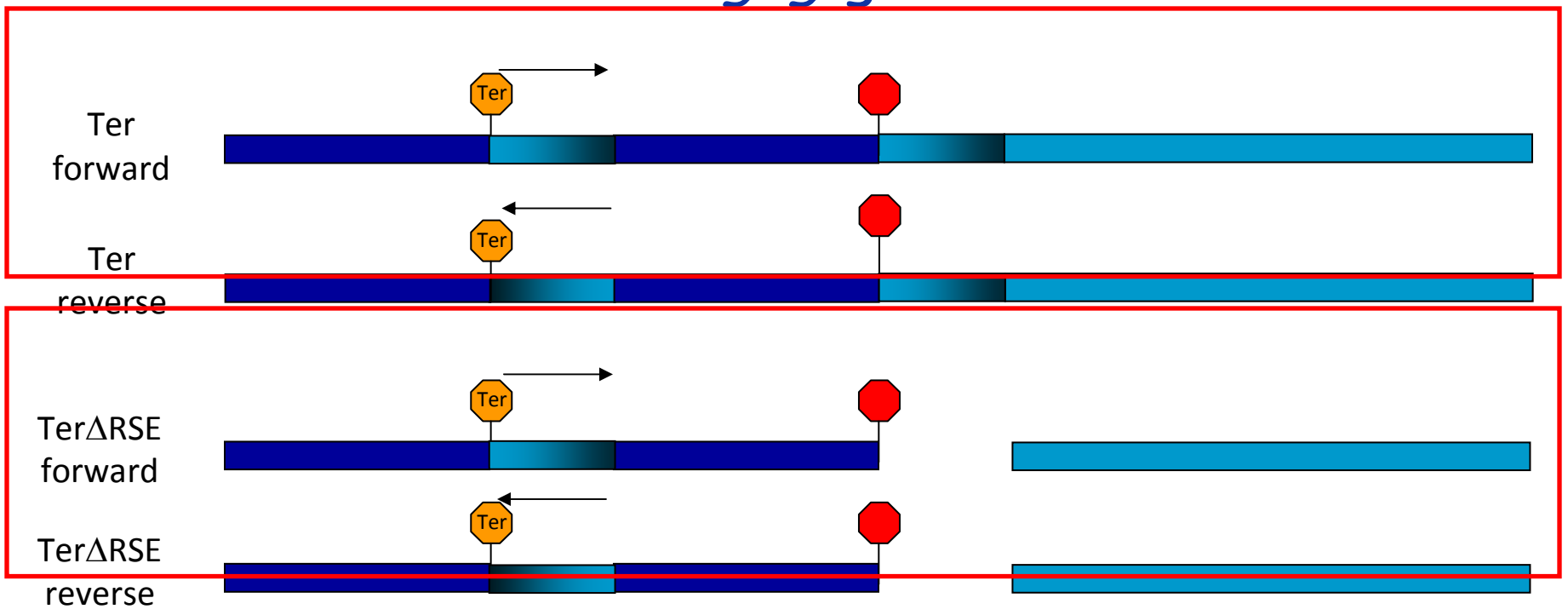
Nonsense codons are usually recognized by downstream exon-junction complexes (EJC) in mammals

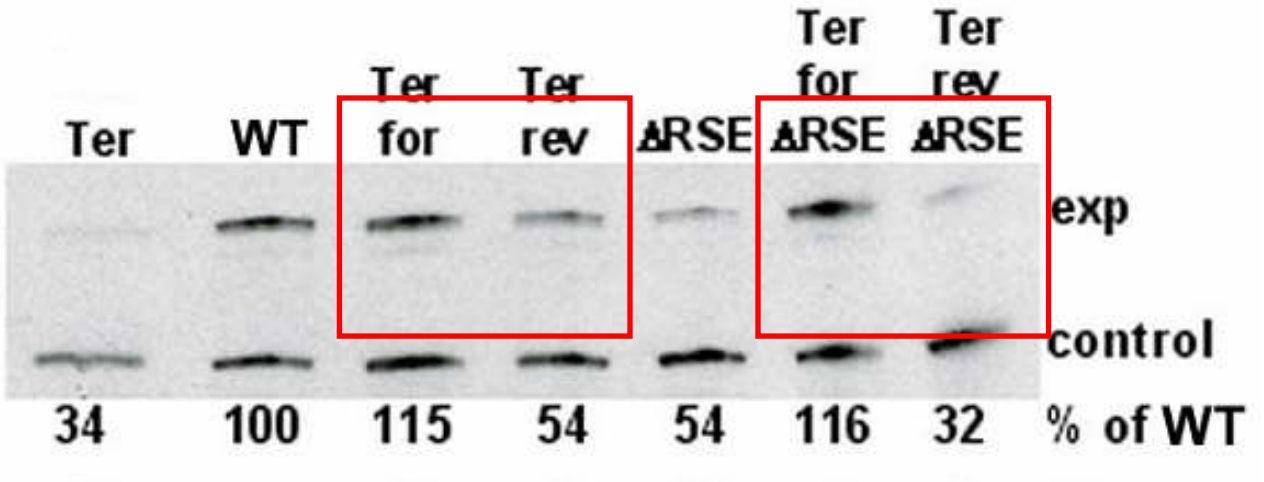


Exon-junction complex (EJC) is a heterogeneous collection of proteins

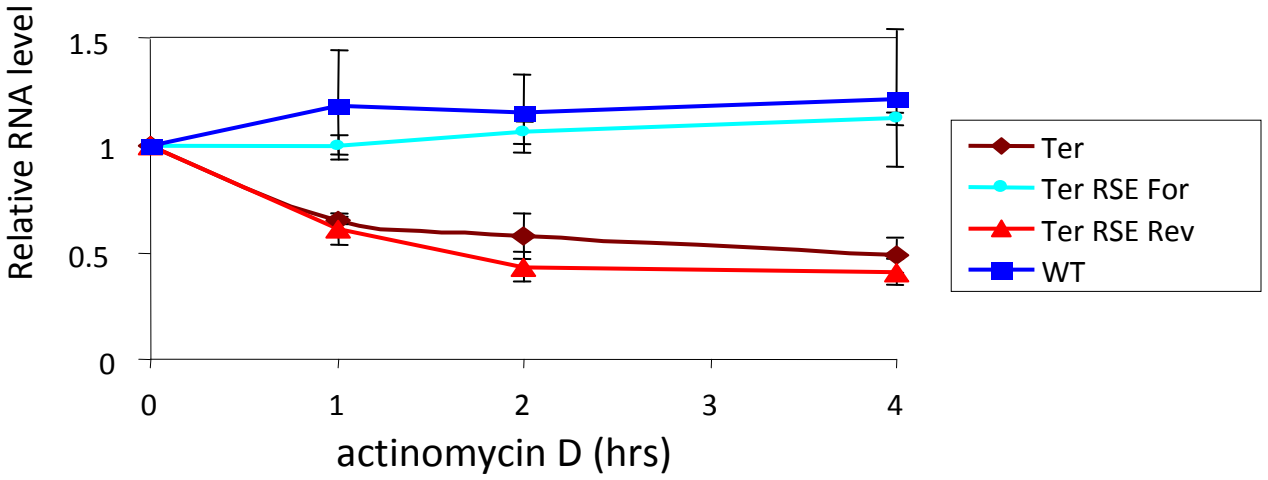


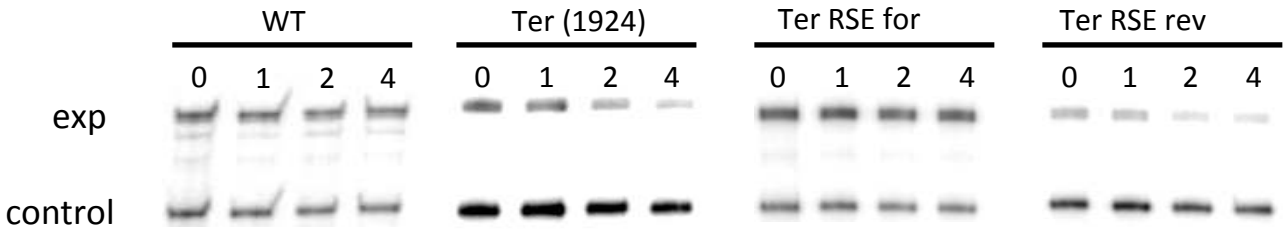
Can the RSE stabilize premature termination within the *gag* gene?



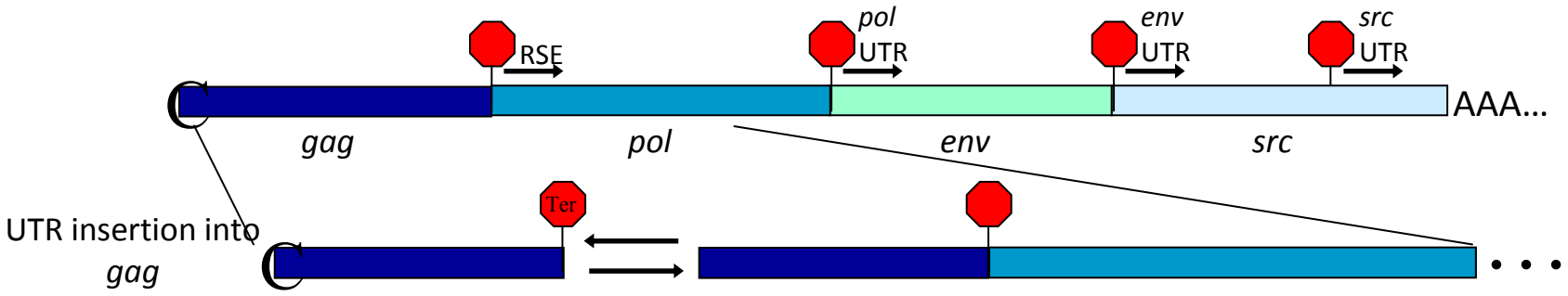


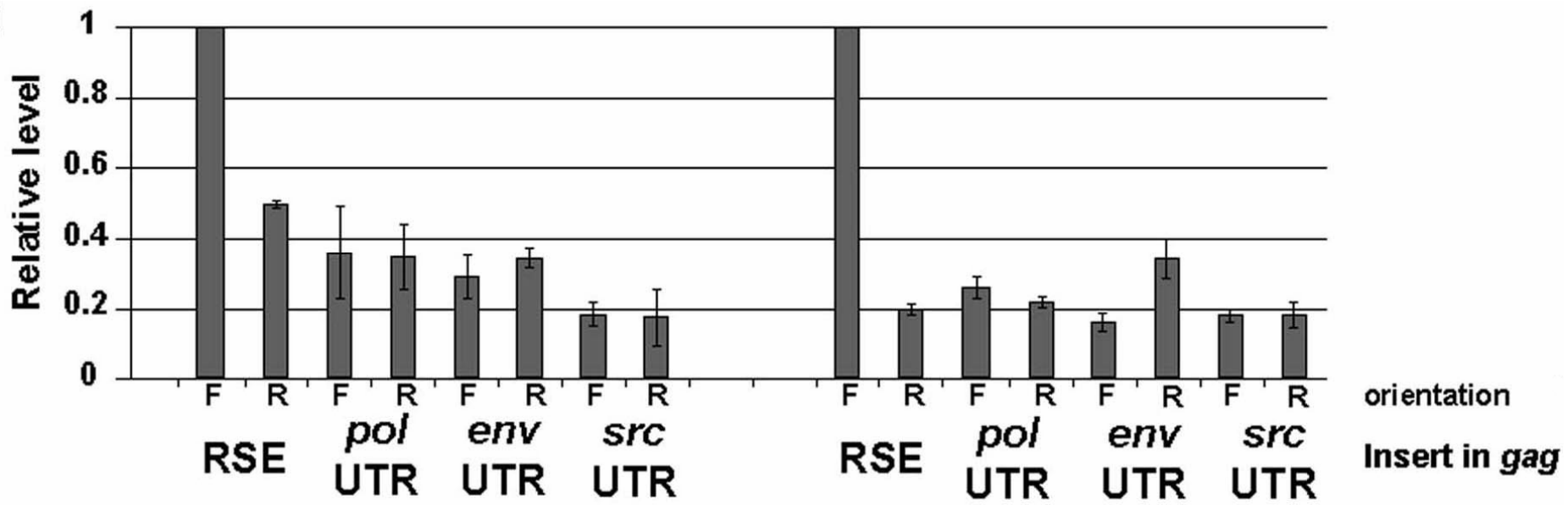
The Stability Element stabilizes PTC-bearing viral RNA





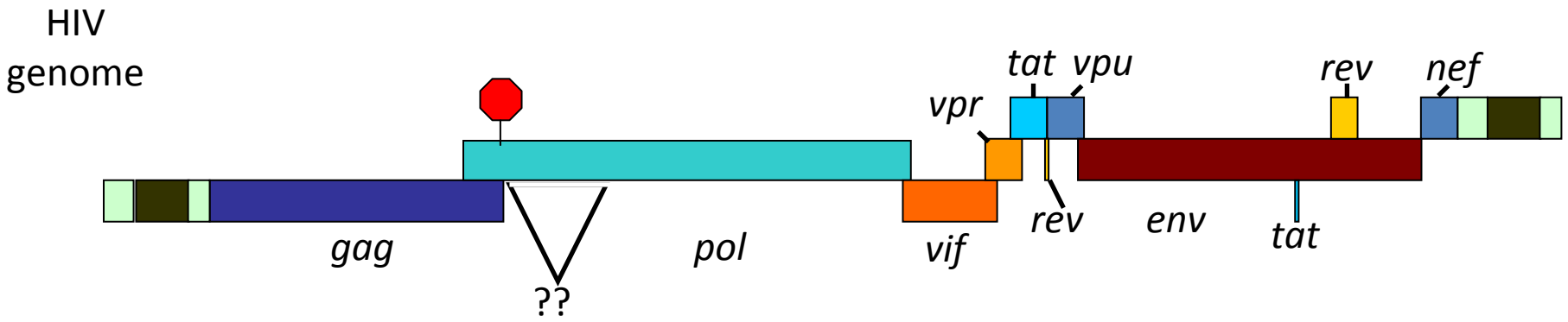
Is there a stability element downstream of other viral genes?

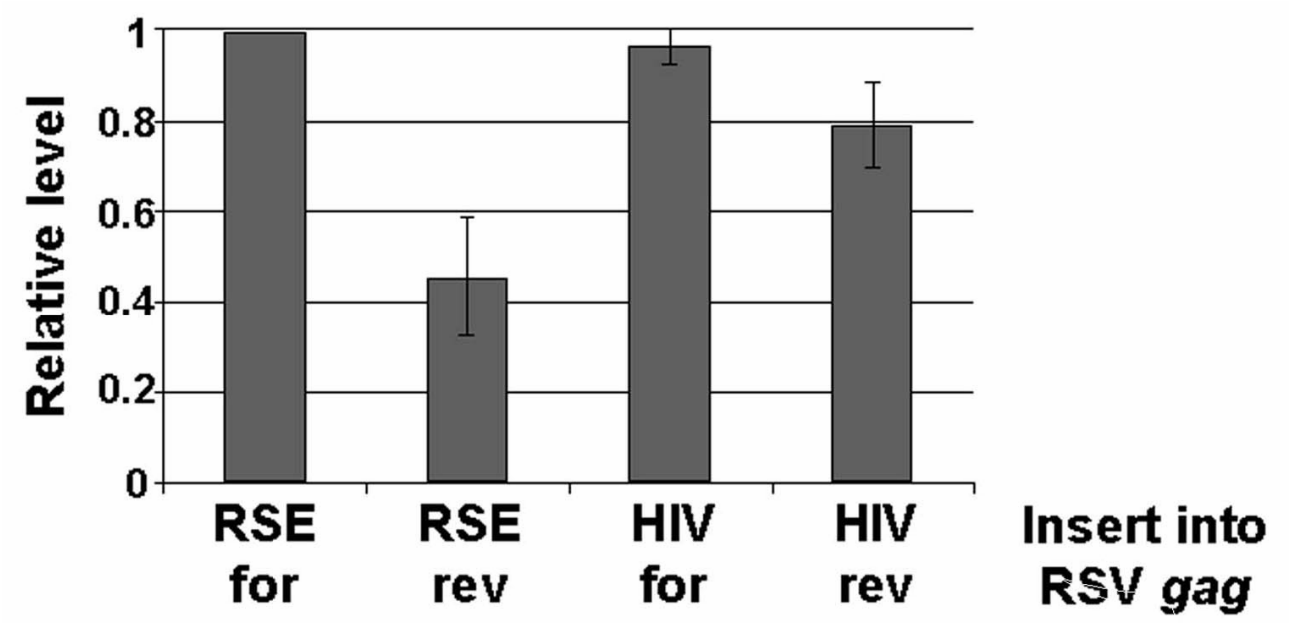




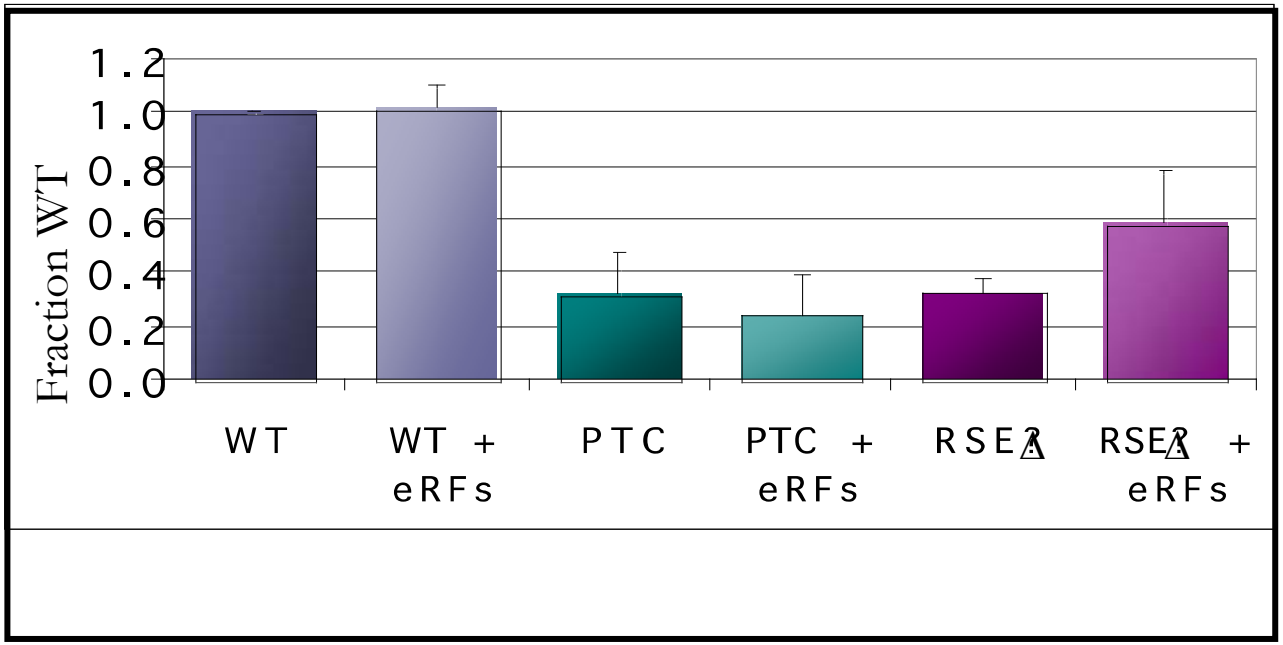
WT ΔRSE

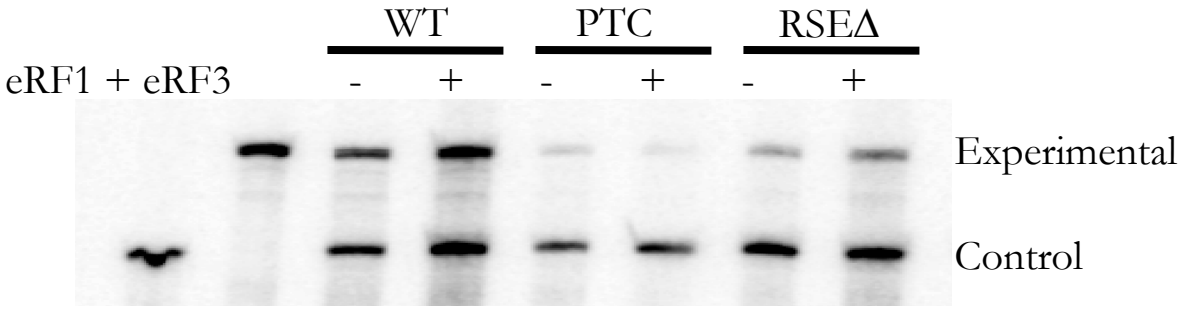
Sequence found after HIV-1 *gag* termination codon stabilizes termination in RSV





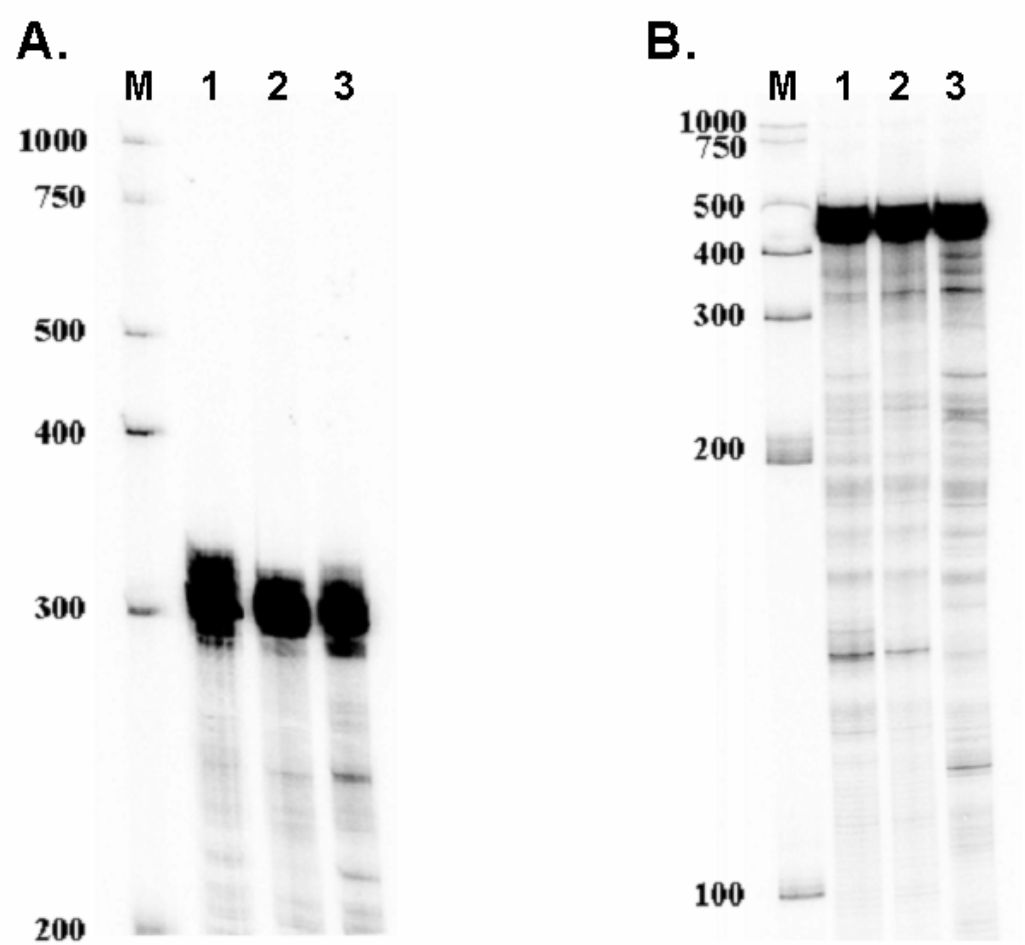
eRF 1 and 3 stabilize ΔRSE RNA, so perhaps RSE promotes termination





Johanna Withers

RSE migrates anomalously in denaturing gels



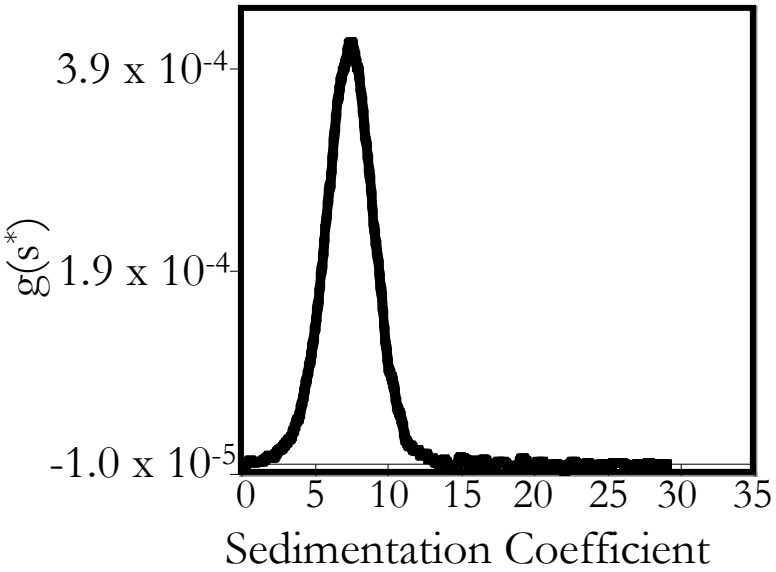
5% acrylamide

10% acrylamide

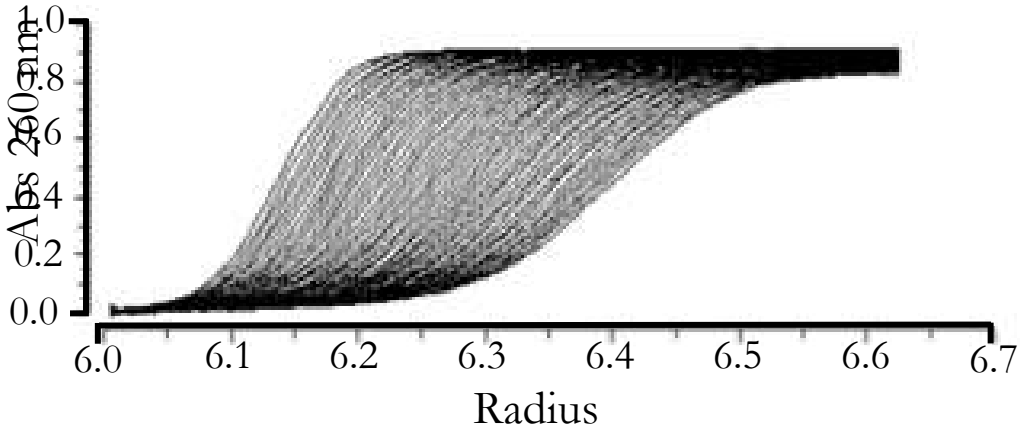
Jason Weil

RSE sediments as monomer in analytical ultracentrifuge

Equilibrium



Velocity



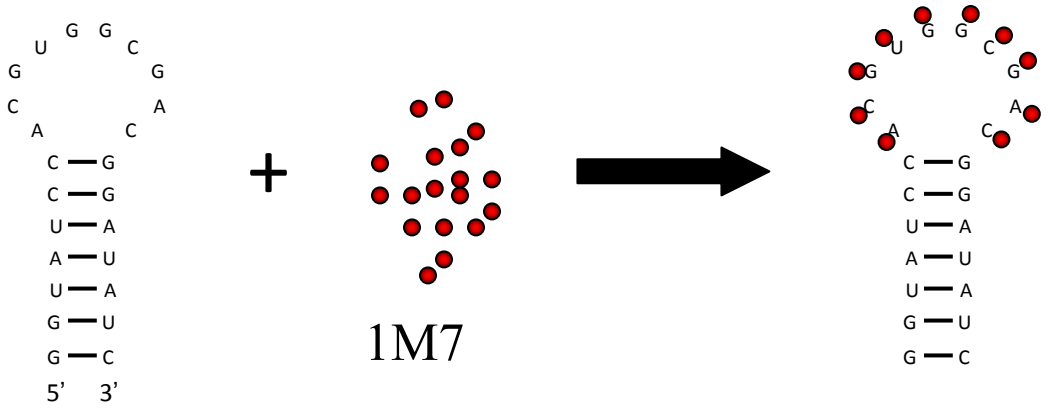
Frictional Ratio = 1.7

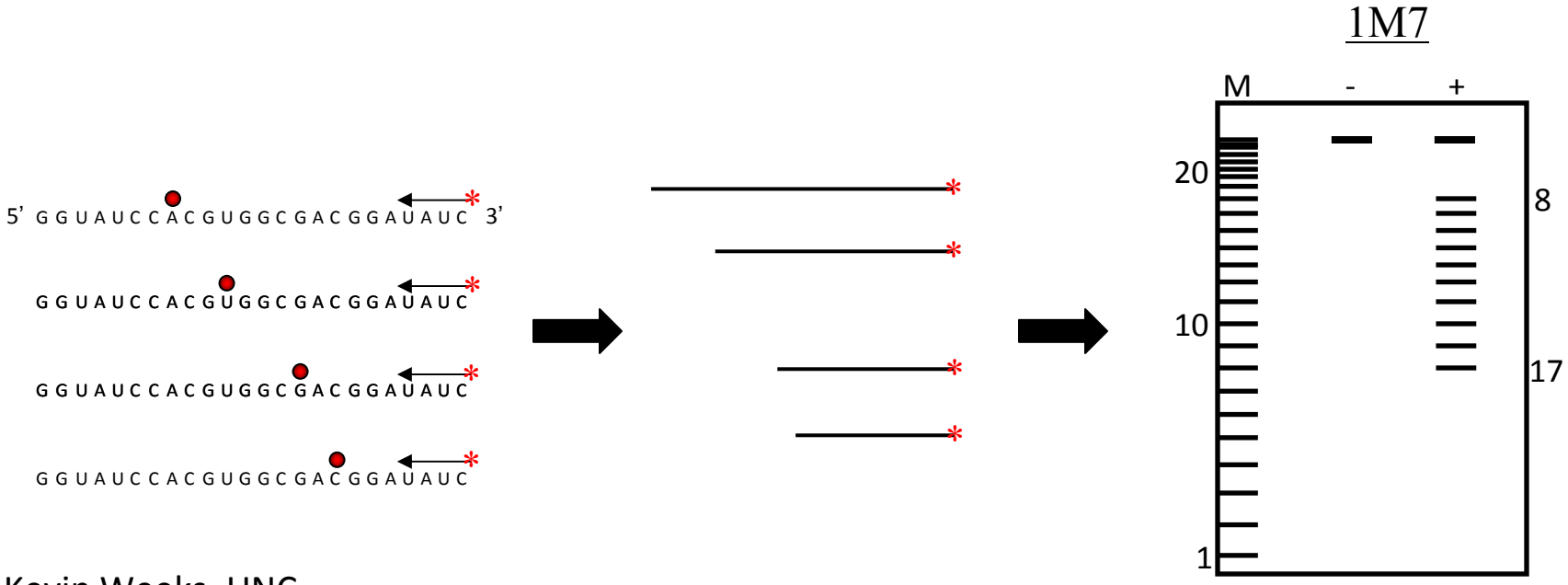
Michael Hadjithomas

Van Moudrianakis

SHAPE chemistry

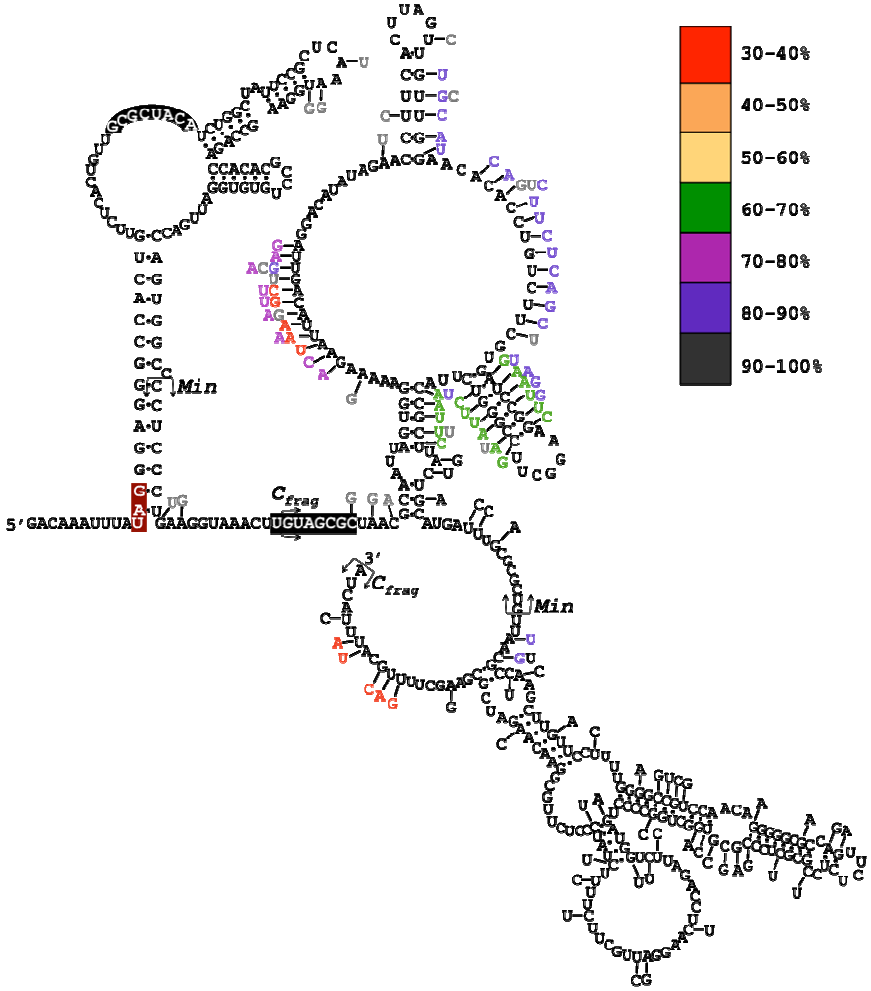
(Selective 2'-hydroxyl acylation analyzed by primer extension)



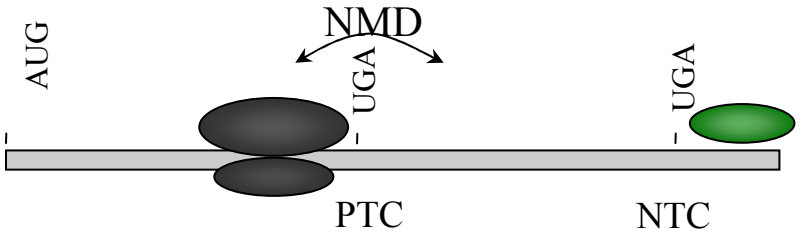


Kevin Weeks, UNC

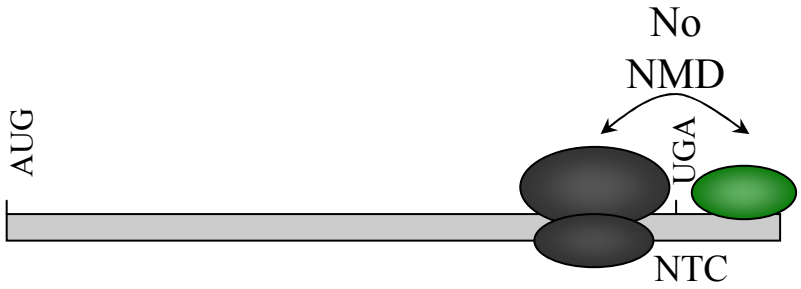
Structure of the RSE



Model: UTR sequences downstream of gag NTC promote proper termination and RNA stability

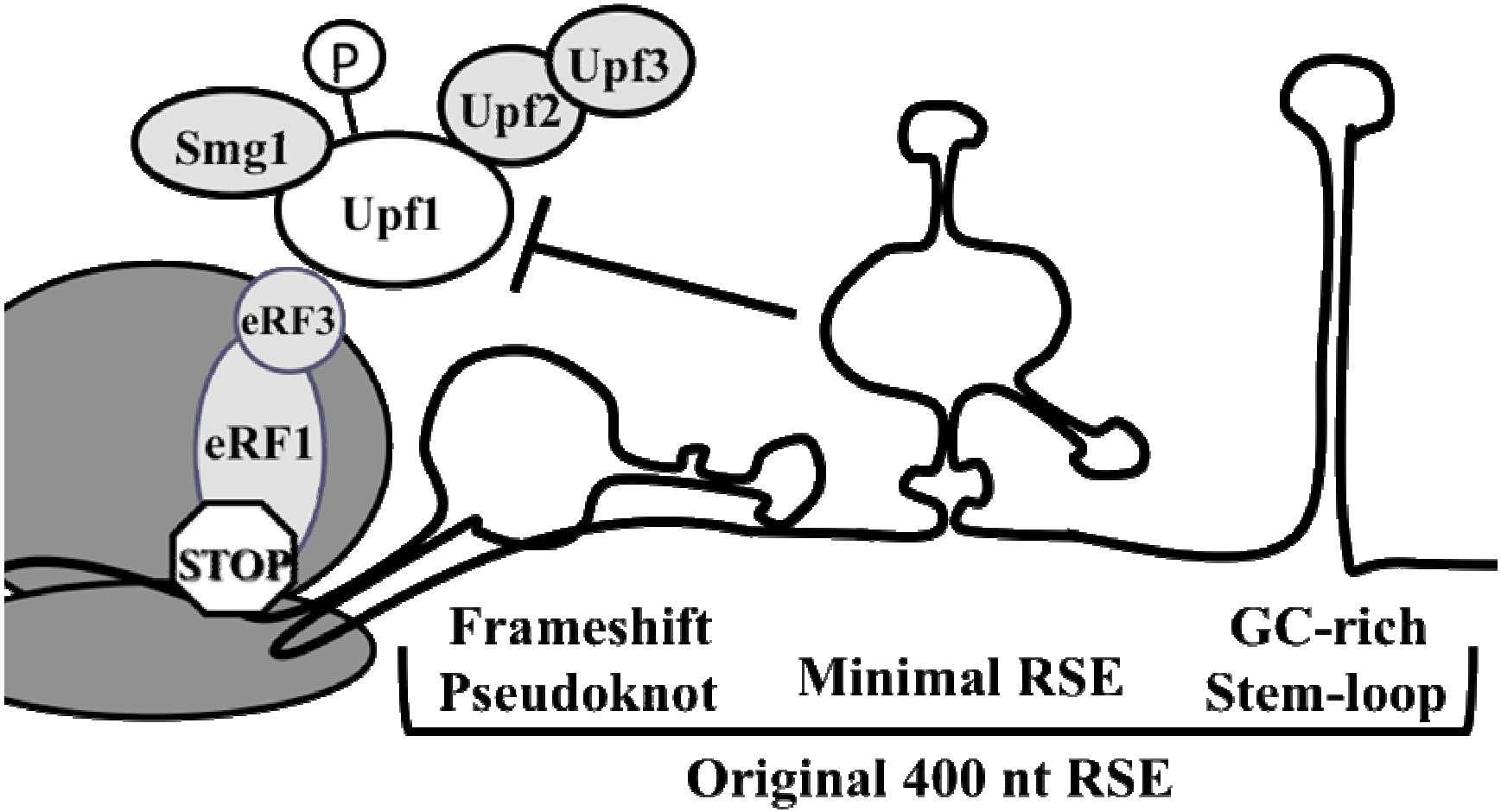


Faux UTR: NMD

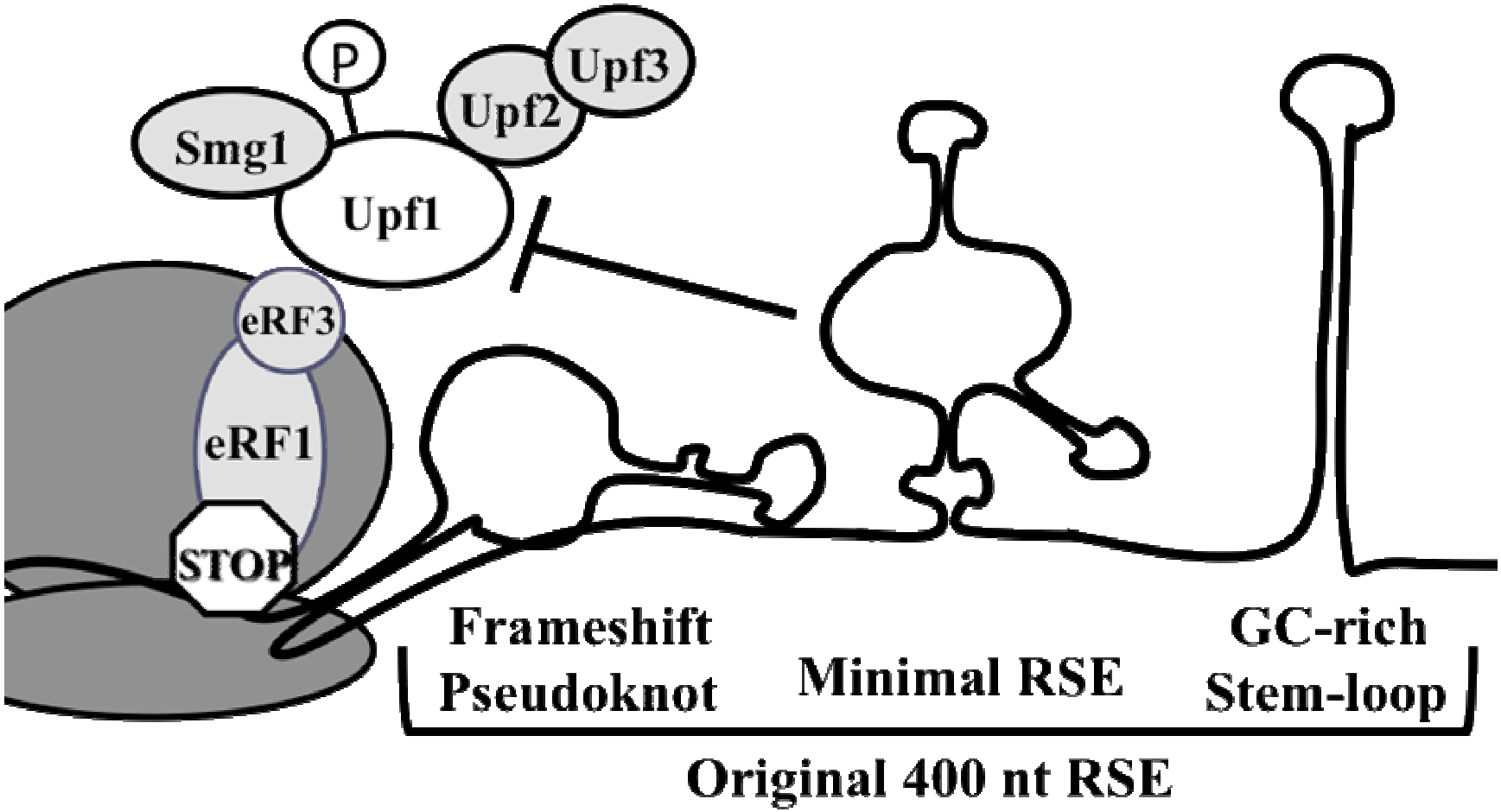


Proper Termination: No NMD

RSE inhibits NMD at *gag* TER

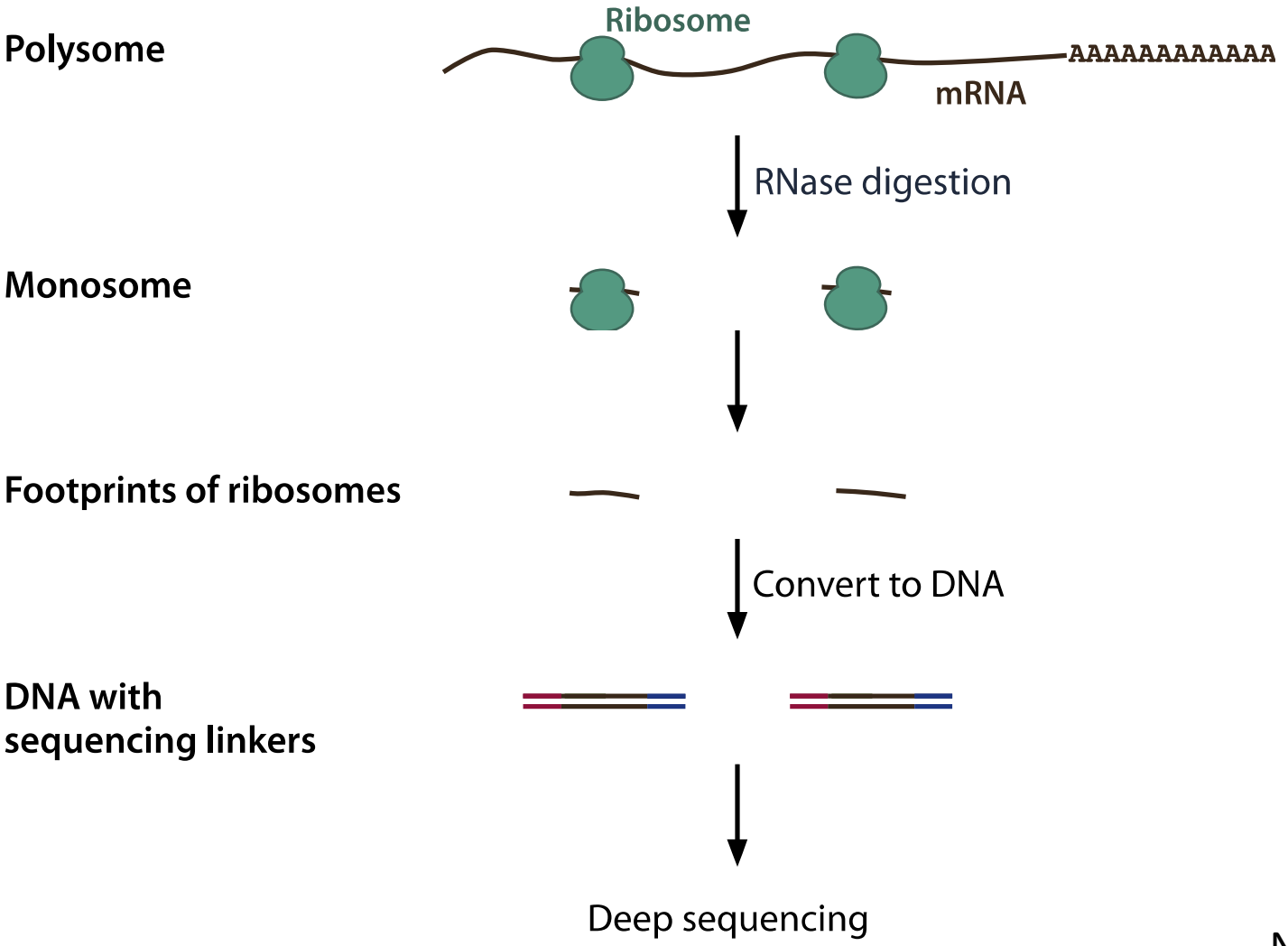


RSE inhibits NMD at *gag* TER



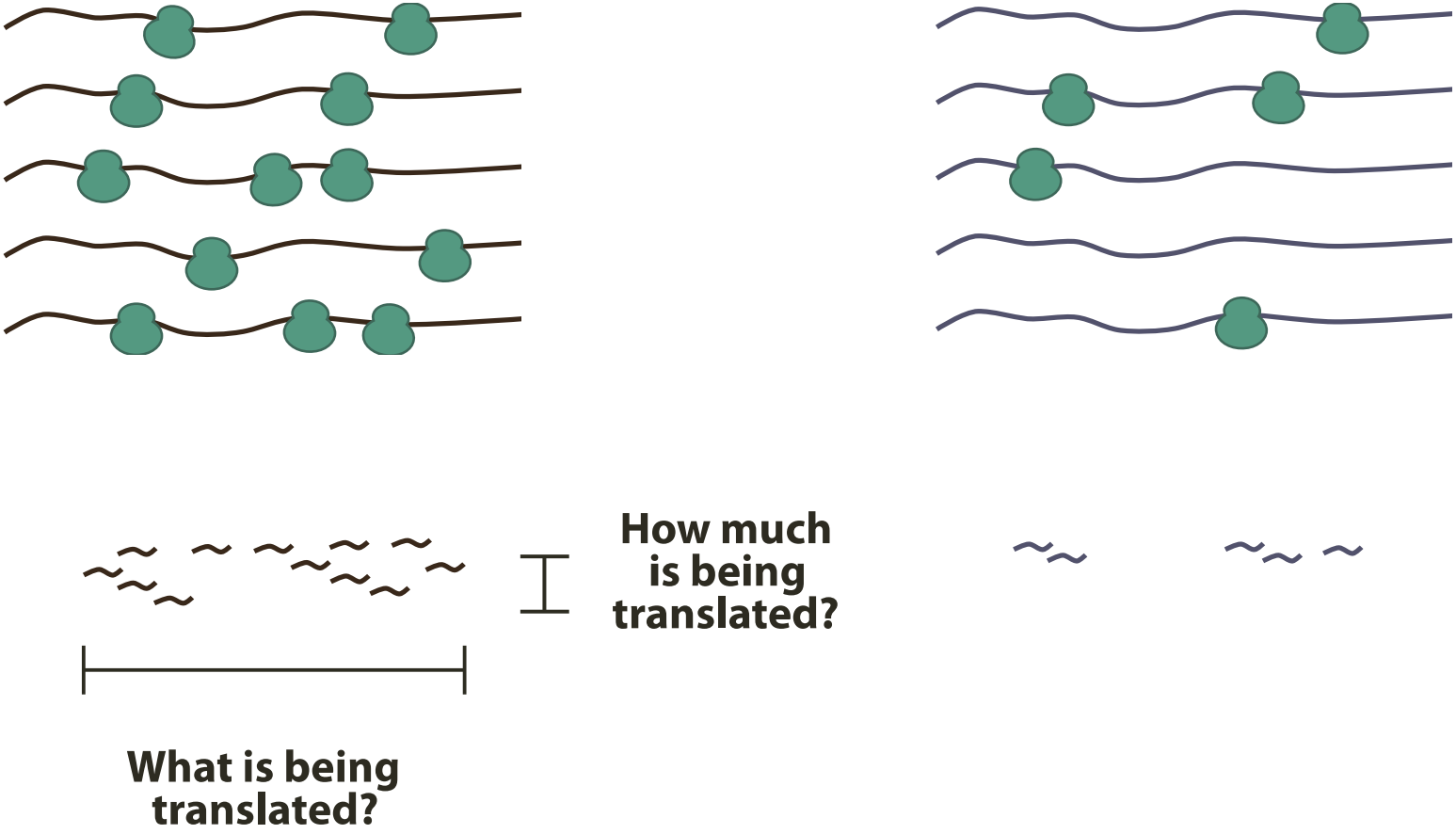
Does it promote translation termination?

Nuclease footprinting of ribosomes on mRNAs indicate *in vivo* ribosome positions

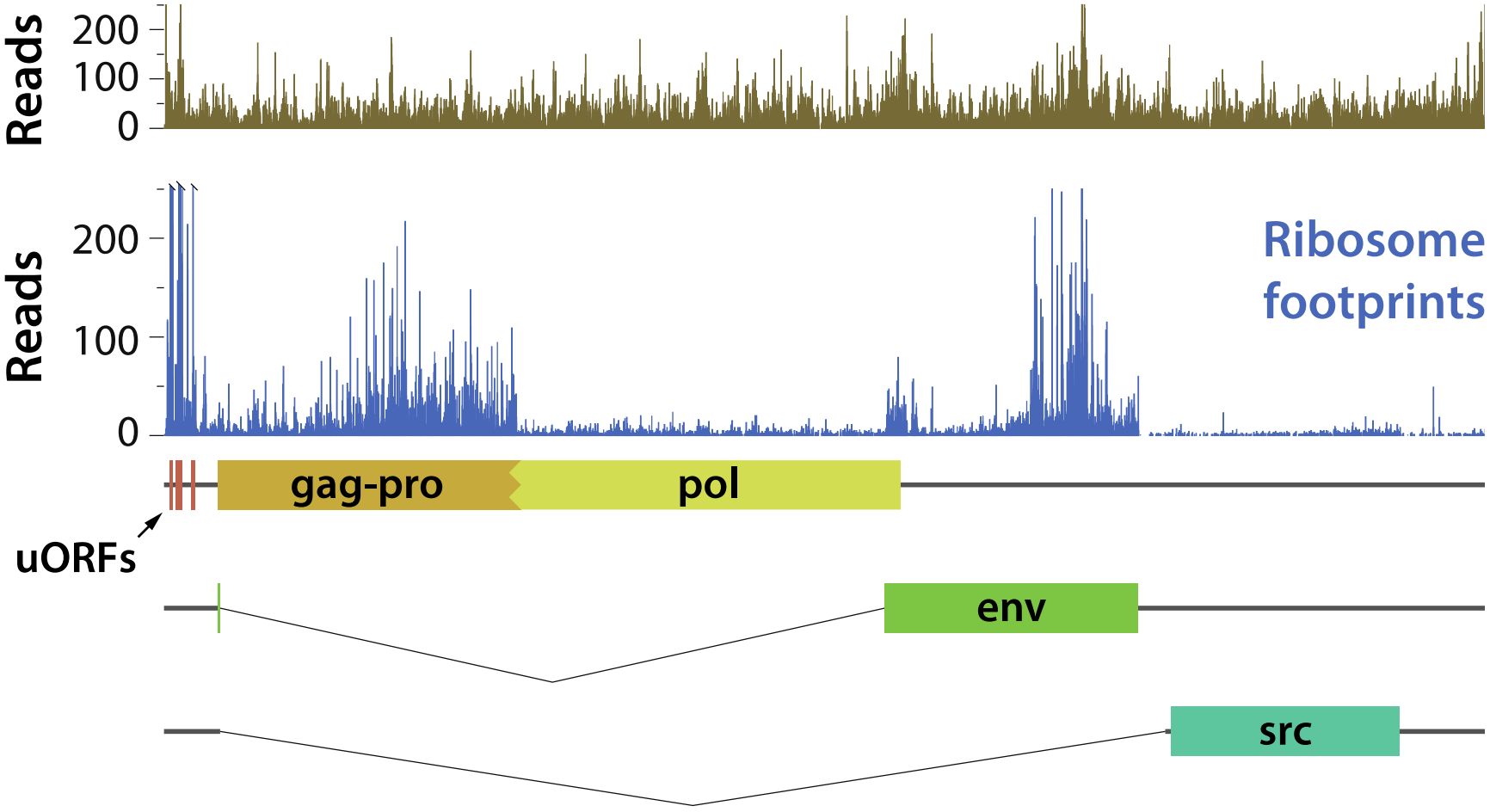


Nicholas Ingolia

Ribosome footprints reveal *in vivo* translation



Ribosome Profile of RSV



CHX treated 1 hr

Summary

- When RSE is deleted, RNA undergoes NMD
- RSE can convert PTC to normal stop codon
- RSE is a highly structured element
- HIV 3'UTR can stabilize an RSV PTC-may be common element in retroviruses or all mRNAs with long 3'UTRs
- Mechanism?
  - Promote termination-interact with ribosome?
  - Interact with Poly (A)/PABP?

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