The HIV-1 antisense RNA *Ast* promotes viral latency via epigenetic silencing of the proviral 5'LTR and is expressed in latently infected cells from ART-suppressed donors

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CONFLICTS OF INTEREST

No conflicts of interest to declare

Key questions:

- Can HIV-1 control establishment and maintenance of latency?
- If so, how does it do that?

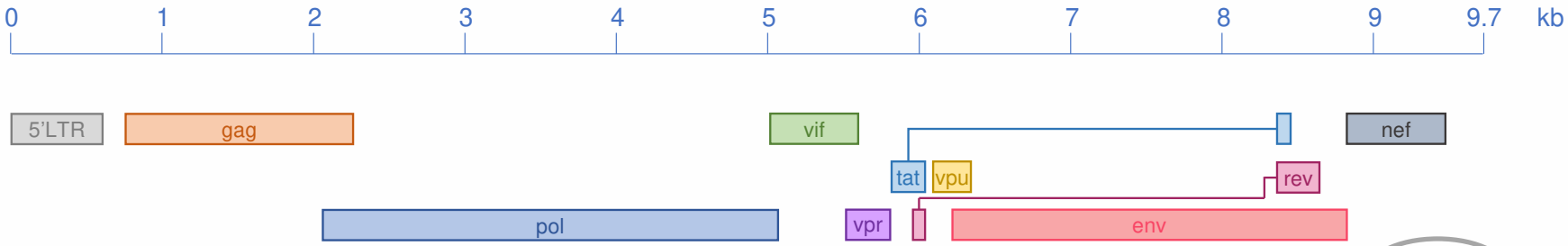
Key findings:

- HIV-1 regulates latency through expression of *Ast*
- *Ast* promotes a closed chromatin state at the 5' LTR

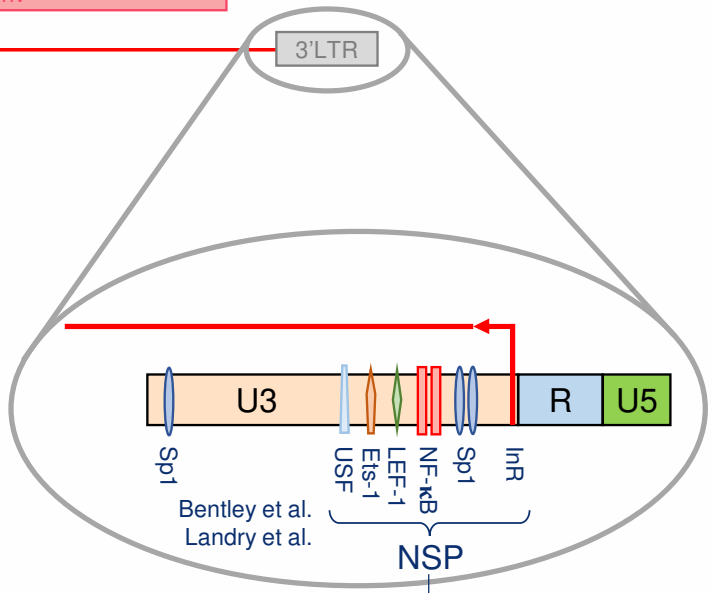
Next steps:

- Exploit *Ast* for "block and lock" and "kick and kill" cure strategies

The HIV-1 antisense gene *asp*



Ast
2574 nt

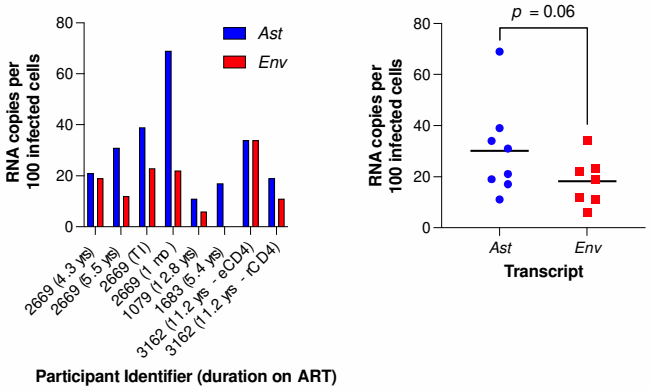


TATA-less, **Tat-independent**
Negative Sense Promoter

CARD-SGS

PID	# infected cells with *env* DNA per 10^6 PBMCs	# infected cells assayed for HIV RNA	# *Ast* RNA sequence	# of infected cells with *Ast* RNA	% of infected cells with *Ast* RNA	*Ast* RNA copies per cell (Range)
2669 (T1)	180	1440	67	63	4.4%	1.1 (1-3)
2669 (T2)	130	1040	54	52	5%	1.0 (1-2)
2669 (T3)	250	2000	86	74	3.7%	1.2 (1-3)
2669 (T4)	90	720	41	40	5.6%	1.0 (1-2)
1079	80	720	43	12	1.7%	3.6 (1-30)
1683	188	1880	22	20	1.1%	1.1 (1-2)
3162 (eCD4+)	3100	496	34	31	6.3%	1.1 (1-2)
3162 (Resting)	2700	4050	40	37	0.01%	1.0 (1-2)

Strand-specific digital RT-qPCR



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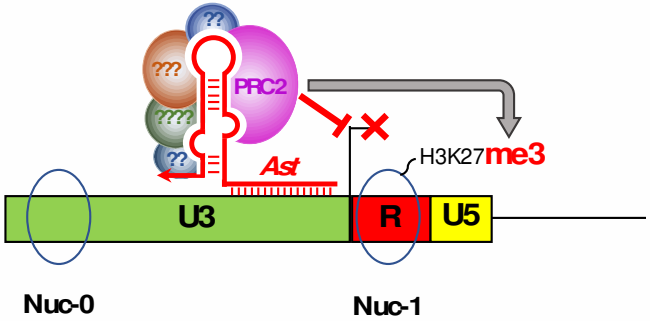
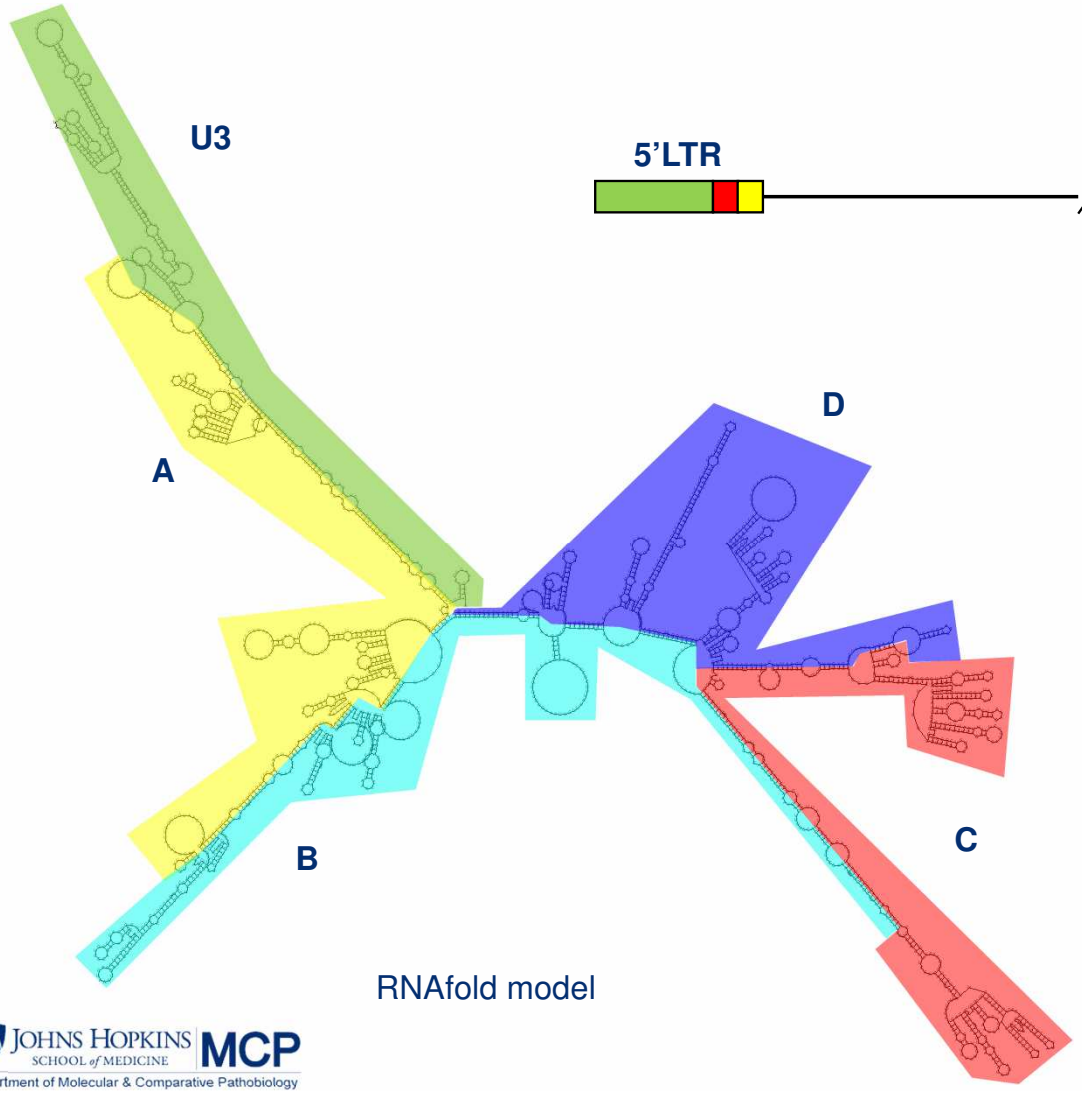
Li et al., Viruses 2021; Gholizadeh et al., Vaccines 2021

The HIV-1 antisense transcript *Ast* promotes latency by recruiting PRC2

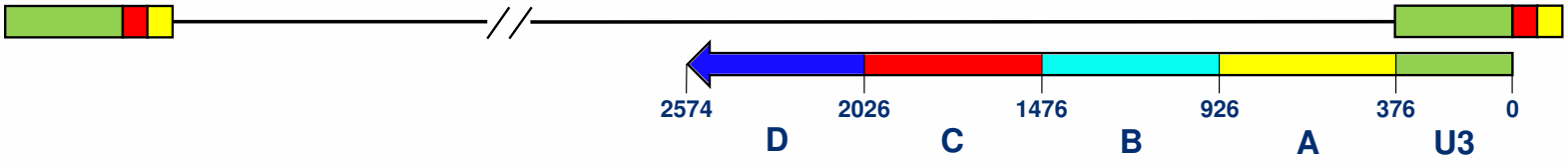
- Over-expression of *Ast* leads to higher levels of PRC2 and H3K27me3 at the 5' LTR
- *Ast* directly interacts with subunits of the PRC2 complex
- Over-expression of *Ast* promotes the establishment and maintenance of latency



Identification of key functional domains and motifs of *Ast*



RNAfold model



Generate *Ast* mutant

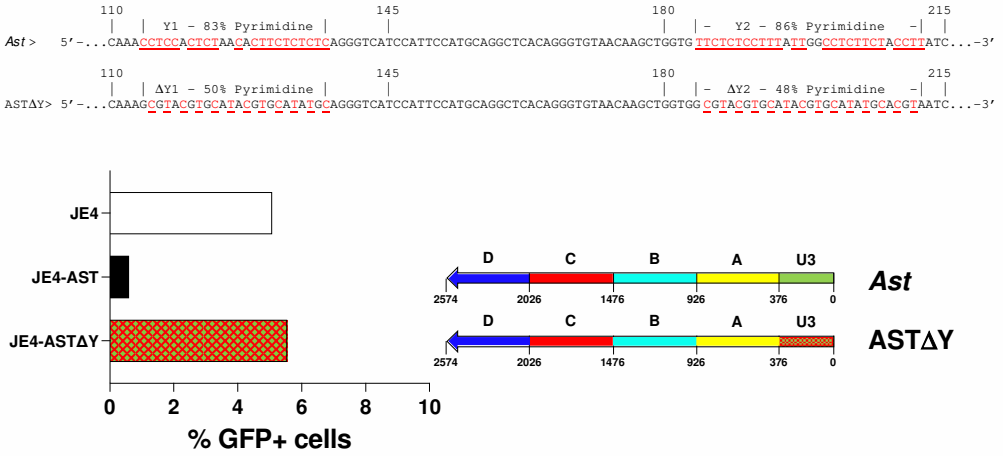
↓

Transduce into JE4 cells

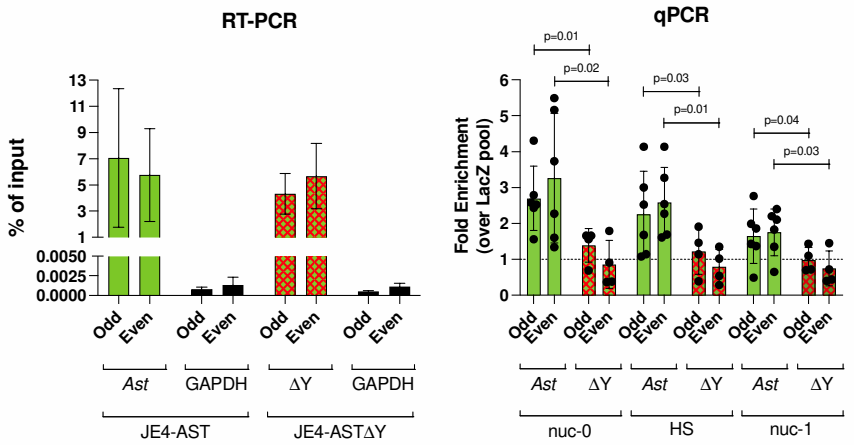
↓

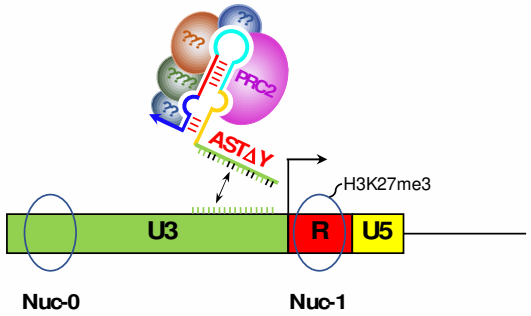
Functional studies (GFP expression)
Molecular studies (ChIP, ChIRP, RIP)

The U3-derived domain of *Ast* interacts with the HIV-1 5'LTR

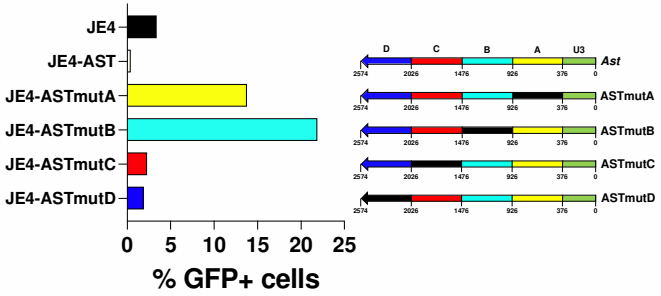


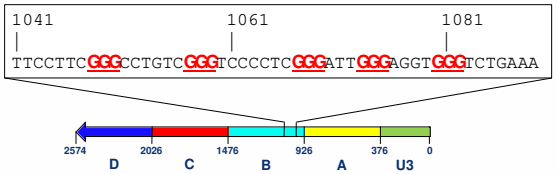
ChIRP assay



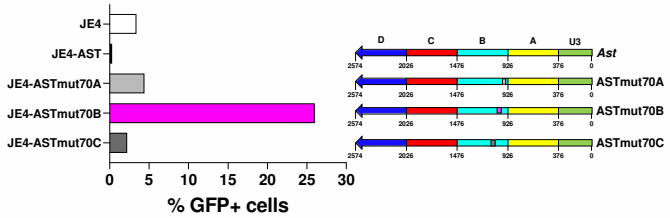


Domain B of *Ast* contains a G-quadruplex motif that binds PRC2

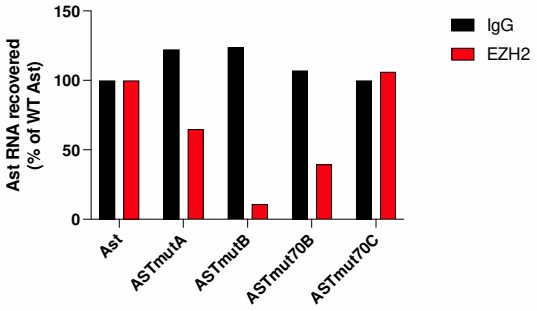




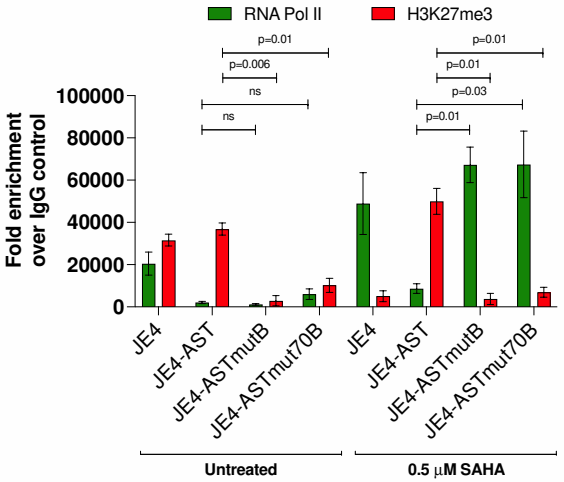
$[G_3N_{3\text{-}7}]_5$



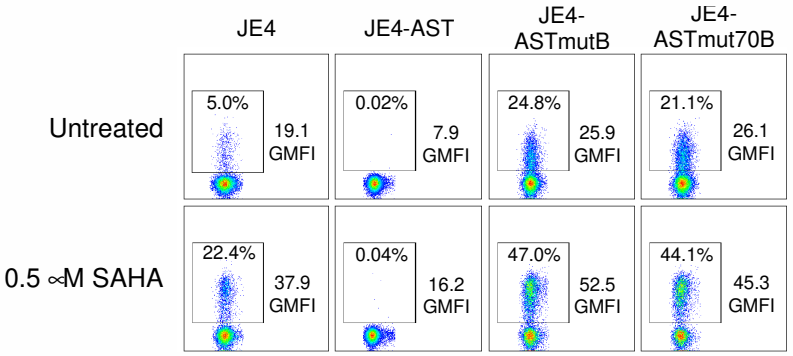
RIP assay

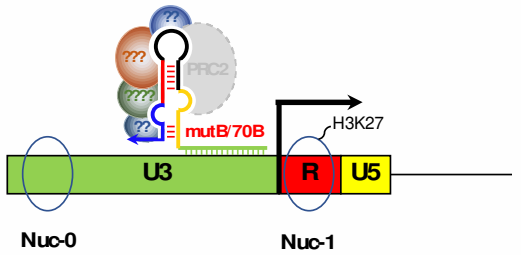


ChIP assay

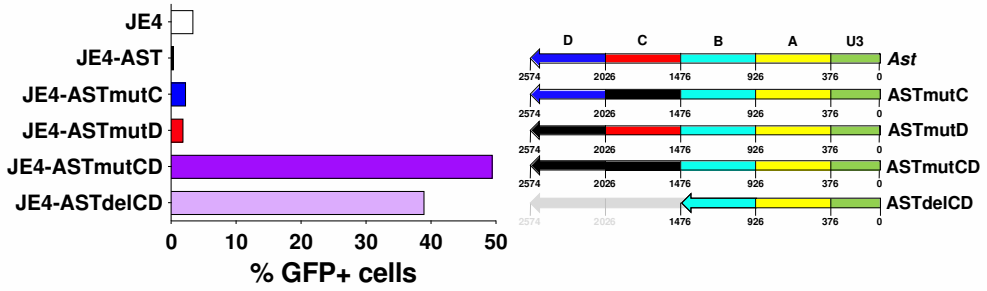


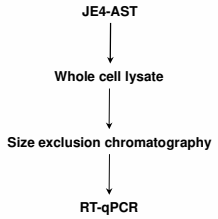
HIV-1 expression

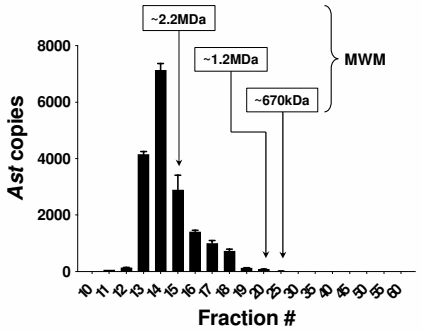




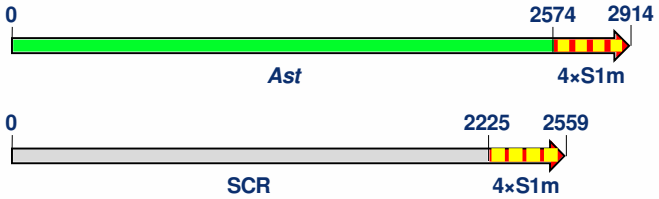
Domains C and D of *Ast* cooperate to recruit epigenetic repressors

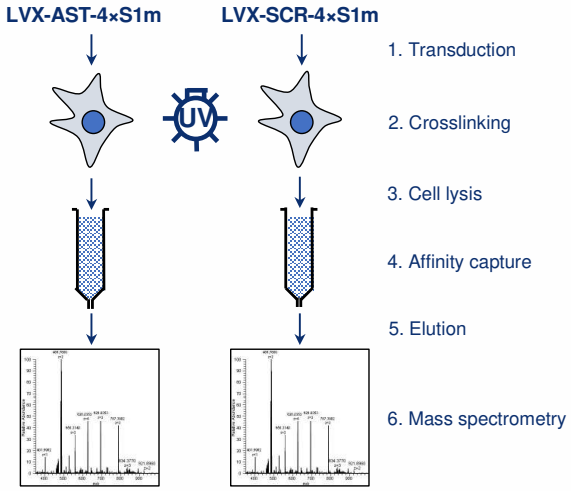






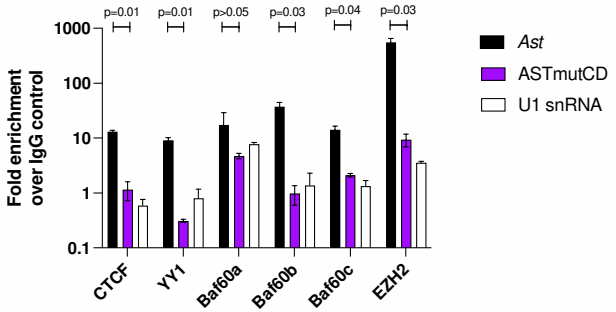
Ast **~830kDa**
PRC2 ~230kDa
??? >1.0MDa





Category	Factor	Ast/SCR	Notes
Epigenetic & Transcription Silencers	BAF60a	15.500	Member of SWI/SNF complex
	YY1	11.724	Transcriptional repressor
	BAF60c	10.339	Member of SWI/SNF complex
	BAF60b	5.370	Member of SWI/SNF complex
	CTCF	4.524	Regulator of chromatin 3D structure
	RCC1	3.837	Regulator of chromatin condensation
	GATAD2B	3.484	Member of NuRD and MeCP1
	HDAC2	3.262	Histone Deacetylase
	TDP-43	2.174	Transcriptional repressor
	RBAP48	2.065	Member of NuRD and PRC2

RIP assay



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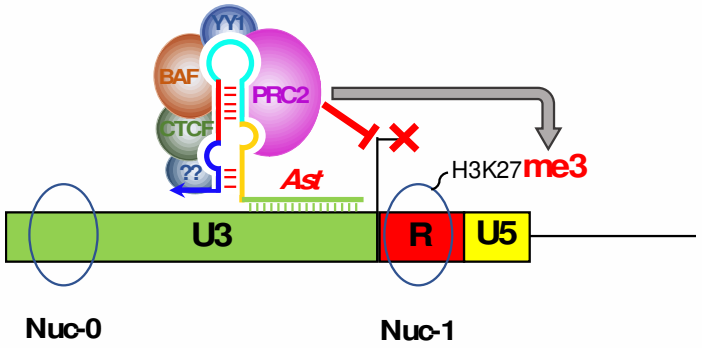
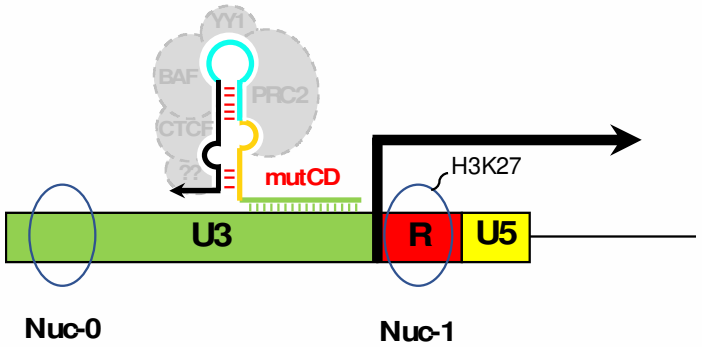
HIV-specific latency induction and reversal by *Ast*





Latency promoting agent

HIV-1 specific

Latency reversing agent

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