A Novel Mode of RNA Binding Assists APOBEC3H in the Molecular Arms Race Against HIV

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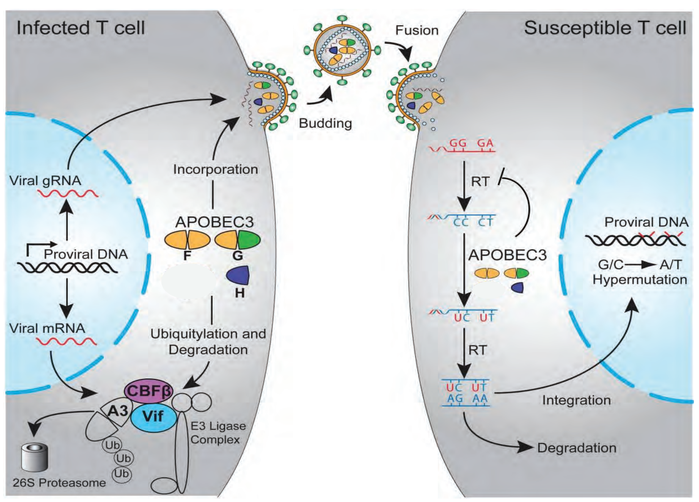
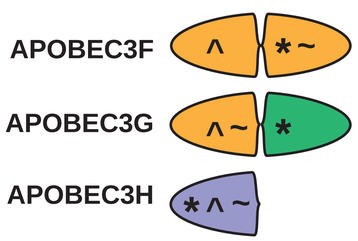
Restriction of HIV-1 by APOBEC3H





A3 biological activities

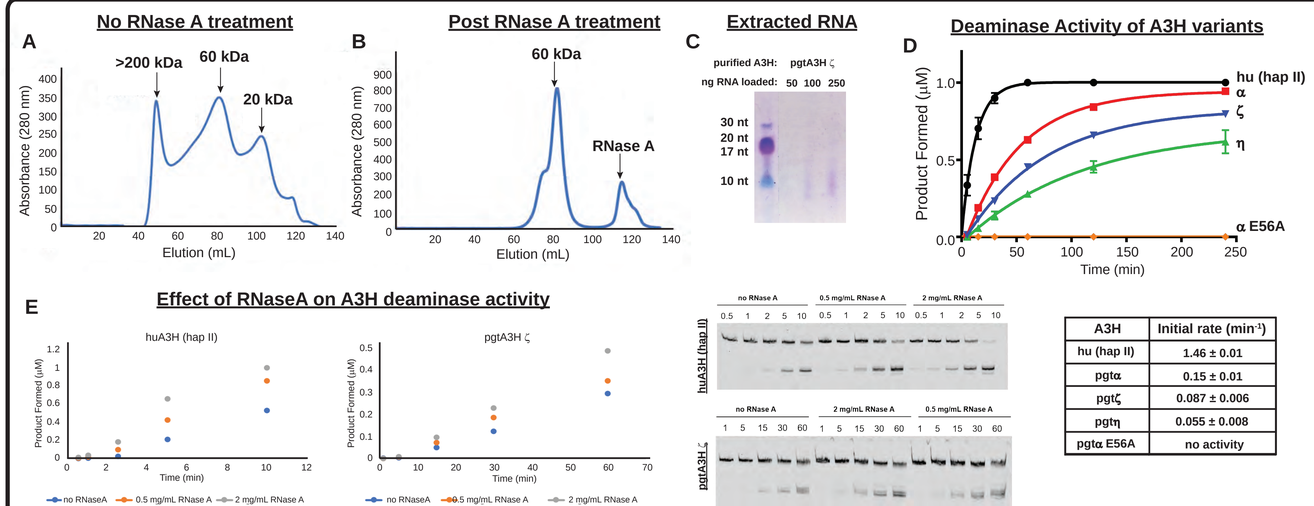
- * Deaminase activity
- ^ RNA binding
- ~ Vif binding

Zinc-coordinating Domains: Z1 Z2 Z3

Zinc-coordinating Motif-
His-X-Glu-X_{23-28}-Pro-Cys-X_{2-4}-Cys
Variance in X positions dictates Z type

Restriction factors in the APOBEC3 (A3) family recognize minus strand (-) DNA (ssDNA) of the HIV-1 genome generated during reverse transcription. This ssDNA is a substrate for zinc-dependent cytidine deamination by resulting in hypermutation and degradation of the HIV-1 genome. APOBEC3 proteins bind to host and viral RNAs to facilitate encapsidation into new virions. HIV-1 Vif counteracts these restriction factors by associating with and recruiting host ubiquitin ligase machinery to APOBEC3 family members A3F, A3G, and A3H, targeting them for proteasomal degradation[1]. While A3F and A3G segregate these functions across two Z domains, A3H uses only one.

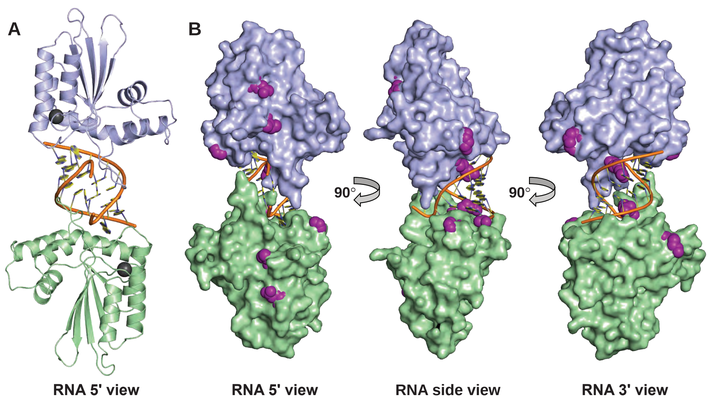
A3H co-purifies with bound RNA and is an active cytidine deaminase



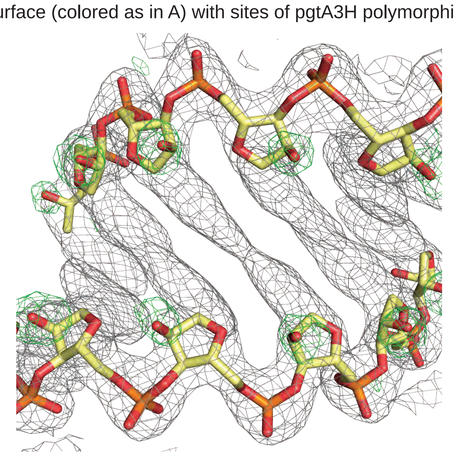
A3H	Initial rate (min^{-1})
hu (hap II)	1.46 ± 0.01
pgtα	0.15 ± 0.01
pgtζ	0.087 ± 0.006
pgtη	0.055 ± 0.008
pgtα E56A	no activity

(A) S200 gel filtration profile of pgtA3Hζ prior to treatment with RNase A, indicating a mixture of oligomeric states. (B) S200 gel filtration profile of pgtA3Hζ after RNase A digestion, indicating a monodisperse dimeric species. (C) Denaturing gel of RNA extracted from the A3H-RNA peak that eluted at 80 mL in (B). The approximate size of the RNA is 10 nt. (D) To assay the cytidine deaminase activity of recombinant A3H variants, I developed a gel-based assay coupled with uracil DNA glycosylase (UDG). Single-stranded fluorophore-tagged DNA (1 μM) containing a single cytosine base was reacted with 100 nM A3H coupled with 40 nM uracil deglycosylase, and products were detected by denaturing gel electrophoresis. Mean and standard deviation of n=3 experiments. (E) The gel based deaminase assay was used to assess the impact of RNaseA treatment on huA3H (hap II) and pgtA3H ζ activity.

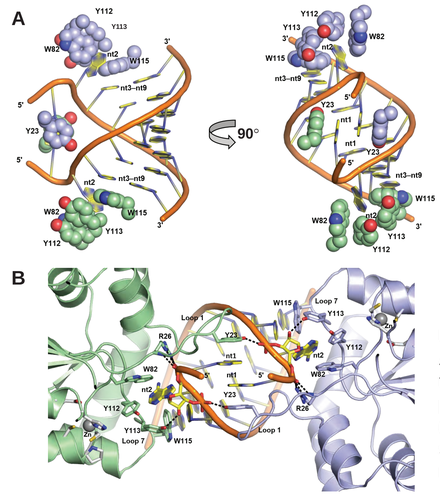
2.24Å Structure of APOBEC3H in complex with duplex RNA



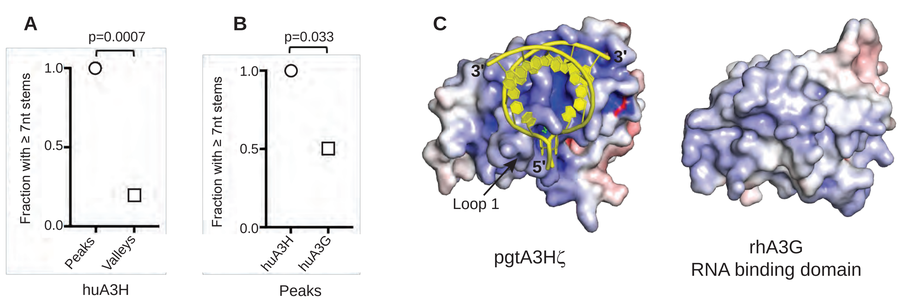
Crystal structure of pgtA3Hζ-RNA complex. (A) Two A3H molecules (blue and green, Zn as gray sphere) at the ends of a central RNA duplex (orange backbone, yellow bases). R_{work}/R_{free} = 0.18/0.22 (B) The A3H surface (colored as in A) with sites of pgtA3H polymorphic variation in magenta.



RNA electron density. 2Fo-Fc density (gray contours at 1.5σ) for the RNA duplex is continuous across each base pair. The Fo-Fc "omit" density (green contours at 3σ), based on a model from which the 2'-OH of each ribose was deleted.

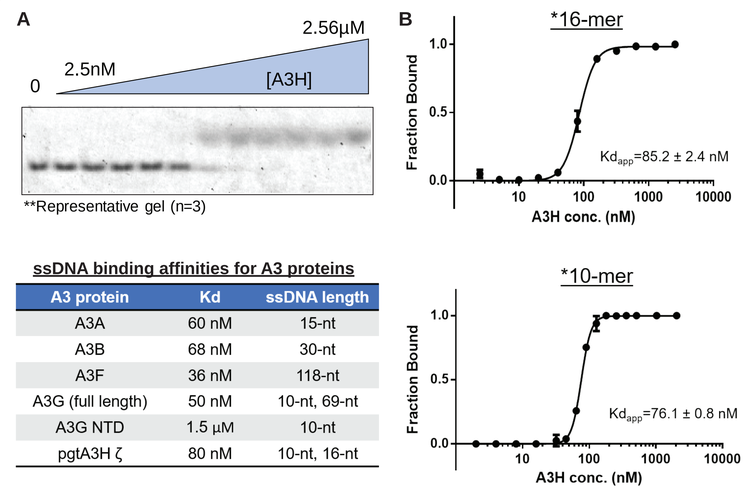


Unique A3H interactions with RNA. (A) Aromatic amino acids (spheres) that contact RNA bases (cartoon). (B) Loop 1 protrusion into the RNA narrow groove. (C) Surface view of one A3H molecule showing the pocket formed by the "aromatic cage" for the unpaired nt2 base.



A3H and A3G exhibit different preferences for duplex RNA binding in virions. CLIP-seq data[2] for A3 binding to the HIV-1 genome were analyzed for the presence of RNA duplex. (A) Frequency of predicted ≥7-bp duplexes within 101-nt RNA elements surrounding the 10 highest-frequency (peaks) and 10 lowest-frequency (valleys) A3H binding sites in HIV-1 virions. (B) Comparison of predicted ≥7-bp duplexes in the sites most frequently bound by A3H and A3G. (C) Surface charge distribution (red to blue, -10 to +10 kT/e) for A3H and the A3G RNA-binding domain (PDB 5K81[3]). The A3H RNA-binding surface is strongly electropositive, but the analogous A3G surface is hydrophobic and forms a dimer (not shown).

A3H binds to ssDNA



ssDNA binding affinities for A3 proteins

A3 protein	Kd	ssDNA length
A3A	60 nM	15-nt
A3B	68 nM	30-nt
A3F	36 nM	118-nt
A3G (full length)	50 nM	10-nt, 69-nt
A3G NTD	1.5 μM	10-nt
pgtA3H ζ	80 nM	10-nt, 16-nt

Electrophoretic mobility shift assays (EMSAs) were performed to determine the apparent binding affinity (Kd_{app}) of A3H for single-stranded DNA (ssDNA). 20 nM 5'-fluorophore labeled ssDNA of either 16- or 10-nt lengths with a central cytosine base was mixed with increasing concentrations (2.5 nM to 2.56 μM) of the inactive A3H variant, pgtA3H ζ E56Q. A representative gel image is shown in (A) and binding curves for the 16- and 10-nt ssDNAs in (B). Comparative analysis of ssDNA binding affinities of several A3 proteins is summarized in the table.

References & Acknowledgements

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3) X. Xiao, S.-X. Li, H. Yang, X. S. Chen, Crystal structures of APOBEC3G N-domain alone and its complex with DNA. Nat Commun 7, 12193 (2016).

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