Towards a Structural Understanding of the APOBEC3F–HIV-1 Vif Interaction

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Abstract:

The human cellular protein APOBEC3F (A3F) is capable of mutating the DNA of the AIDS virus HIV rendering it non-functional and incapable of replication(1). A3F enters virus particles during reverse transcription and converts viral cDNA cytosines to uracils, which template the insertion of adenines instead of guanines and result in strand-specific mutations. However, A3F does not normally have a chance to be effective because it is antagonized by a small HIV-1 protein called Vif, which that triggers the degradation of A3F(2). Nevertheless, the A3F mutagenic signature is still found within the DNA of the HIV-1 virus itself, indicating that the anti-viral activity of this protein can manifest in vivo (in people) and that therapeutics that stimulate this activity may be beneficial. We hypothesize that a high-resolution structure of A3F will provide crucial information that will enable the rational design of compounds that disrupt the A3F-Vif interaction. To achieve this goal, we first identified the minimal region of A3F that is both catalytic and able to bind Vif. Second, we improved the activity of this construct using structure-guided mutagenesis. Third, we are presently optimizing purification conditions to improve solubility.

Introduction :

APOBEC3F is capable of inhibiting Vif deficient HIV in vivo (1).

Vif is a small protein which is part of the HIV reverse transcriptase machinery and acts as an accesory factor for reverse transcription.

We performed mutagenesis to find the minimal domain of A3F which is able to bind Vif and also catalytic (Harris Lab, unpublished data).

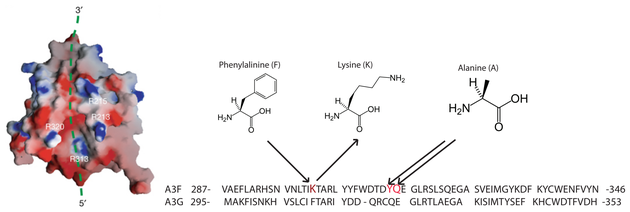
Harris Lab members performed structure directed mutagenis to optimize the catalytic activity of A3F, using the structure of APOBEC3G (A3G) as a guide.

Currently we are performing rifampicin-resistance (Rif^R) mutation assays (3) to screen for *E. coli* strains which produce a folded soluble A3F protein.

NMR or X-ray crystallography will then provide a high resolution structure of A3F.

Active Protein Search:

Structure guided mutagenesis was performed to find the minimal domain of A3F which is able to bind Vif and also catalytic. Harris Lab members used the structure of APOBEC3G (2) to find key homolgous residues which can alter the solubility and activity of the APOBEC3F protein. Point mutations were made accordingly and A3F-CTD with the mutations F302K, A314Y and A315Q (A3F-2K3A) is currently the most catalytic A3F.

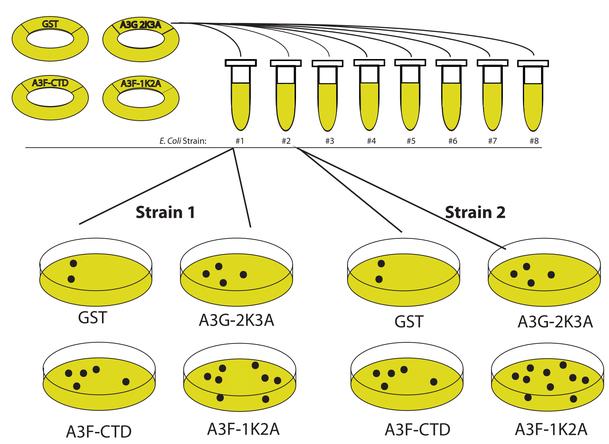


Structure of the APOBEC3G protein (4) as well as the homologous domain from APOBEC3G. Point mutations were made based on this data.

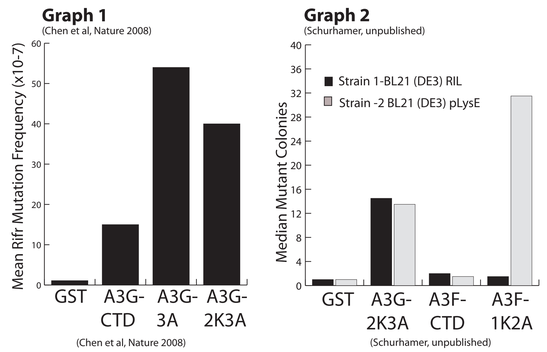
Soluble Protein Search:

A rifampicin-resistance mutation assay has been used extensively to monitor the intrinsic DNA cytosine deaminase activity of several A3 proteins including A3F. Here we show a published result (Chen et al, Nature 2008) and how an increased mutation frequency, represented by greater aquired Rif resistant phenotype showed a positive correlation with protein solubility.

1. Each *E. Coli* strain was transformed with all 4 plasmids and grown overnight

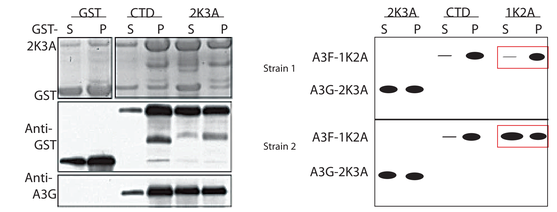


2. Overnight growths placed on LB+Rifampicin plates and grown over night.



3. Mutation frequencies of *E. coli* strains in Rif^R assay. At left, mutation frequencies of A3G (4). At right, mutation frequencies as indicated by median colony frequency in two strains of *E. coli*.

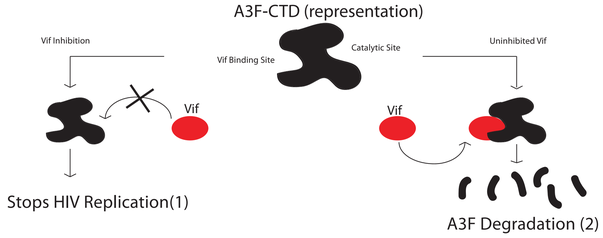
Soluble Protein Search:



4. At left solubility of GST, GST–A3G-CTD and GST–A3G-2K3A, as monitored by SDS–PAGE and coomassie blue staining (top panels) or immunoblotting (anti-GST middle panel and anti-A3G bottom panel). (4). At right, an anticipated SDS-PAGE gel with increased solubility for Strain 2 as indicated by an larger supernatant (S) to pellet (P) ratio. This result is predicted due to the high frequency of Strain 2 in the rifampicin-resistance mutation assay.

Vif Inhibitor Search:

Attain a high resolution structure of A3F using NMR or X-ray crystallography.



Summary and Future Directions:

APOBEC3F intrinsic deaminase activity can be quantified for activity and solubility using rifampicin-resistance mutation assays. The soluble protein produced can then be used for GST-pulldown experiments, NMR spectroscopy, X-ray crystallography and further mutagenesis.

An understanding of the A3F- Vif interaction will provide key insight into designing a drug which may cure HIV-1.

References:

1. Liddament et al. 2004. APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. Curr Biol 14:1385-91.
2. Conticello et al. 2003. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr Biol 13:2009
3. Harris, R.S. et al 2003. DNA deamination mediates innate immunity to retroviral infection. Cell 113, 803–809
4. Chen et al. 2008. Structure of the DNA deaminase domain of the HIV restriction factor APOBEC3G. Nature 452:116-9.
5. Russell et al. 2008. Distinct domains within APOBEC3G and APOBEC3F interact with separate regions of HIV-1 Vif. J Virol, in press.

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