Characterizing Novel N-terminal Domains of APOBEC3G

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ABSTRACT

Background: The human cytidine deaminase APOBEC3G (A3G) has been shown to exhibit antiviral function in the absence of its established catalytic function. A thorough molecular understanding of this deamination-independent mechanism is largely unknown.
Methodology: We sought out to characterize the A3G protein and identify key regions and domains related to its antiviral function. A library of mutants was constructed and these mutants were examined for antiviral function in a *vif*-deficient system. 293T cells were transiently co-transfected with proviral DNA and the mutant construct of interest. A3G and viral expression was verified by Western blotting. Viral particles were quantitated, normalized and then used to infect TZM cells in a single round infectivity assay. Finally, virions were purified and assessed for A3G packaging. Concurrently, the catalytic activity of the mutants was examined using an antibiotic-resistance based *E.coli* assay. Concurrently, the subcellular localization of the mutant A3G proteins was examined.
Results: We have identified two novel N-terminal domains that upon mutation confer a significant loss of antiviral function, but not an observable decrease in catalytic function.
Conclusions: These novel domains are quite interesting because they appear to play a significant role in antiviral function but not catalytic function. We are currently looking at both the mehanism behind these observations as well as the evolutionary conservation of these residues to determine whether these identified domains fall within regions of the A3G protein that have been subjected to episodes of positive selection.

CATALYTIC ACTIVITY

A3G construct	Antiviral Activity (%)	Catalytic Activity (%)
wildtype	100	100
E259Q	97	6
Mt16	53	81
Mt67	21	79
Mt68	22	55
Mt33	41	117
Mt51	10	73
Mt52	17	121
Mt53	12	113

Table 1: Mutants were analyzed for catalytic activity using a rifampicin-resistance based *E.coli* assay. Mutants were found to retain catalytic activity but were unable to inhibit viral replication. These data support a deaminase-independent mechanism of A3G antiviral activity.

INFECTIVITY

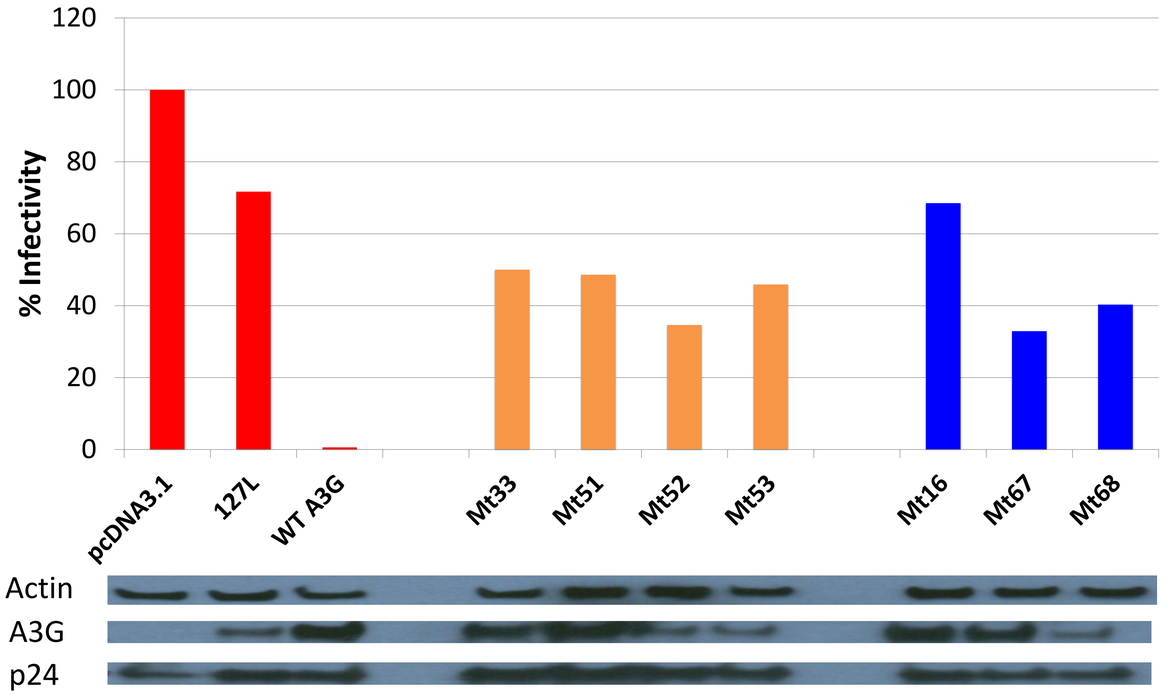


Figure 1: Antiviral activity of mutant A3G proteins. HIV-1 was co-transfected into 293T cells with the A3G mutant construct of interest or the vector (pcDNA 3.1). Virus-containing supernatants were harvested at 48 hours, normalized and used to infect TZM reporter cells. Data are normalized to the vector control. A representative experiment is shown. A3G and p24 expression in 293T cells was determined by western blotting.

SUBCELLULAR LOCALIZATION

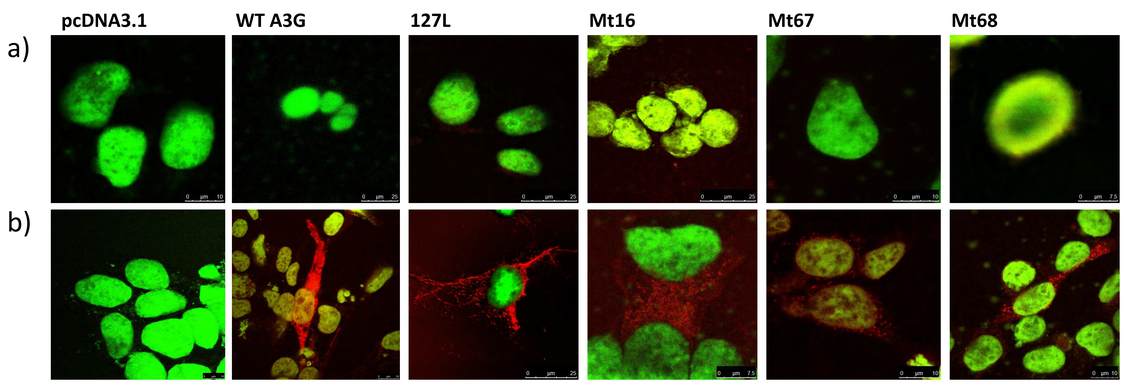


Figure 2: Subcellular localization of A3G determined by immunofluorescence. Nuclei were stained with Sytox green and A3G is stained with an Alexa 633-conjugated secondary antibody. **a)** 293T cells were co-transfected with HIV-1Δ*vif* proviral DNA and A3G construct. Cells were incubated secondary antibody *only*.[This serves as a control for nonspecific binding of the secondary antibody] **b)** Cells were incubated with both primary and secondary antibodies.

VIRION PACKAGING

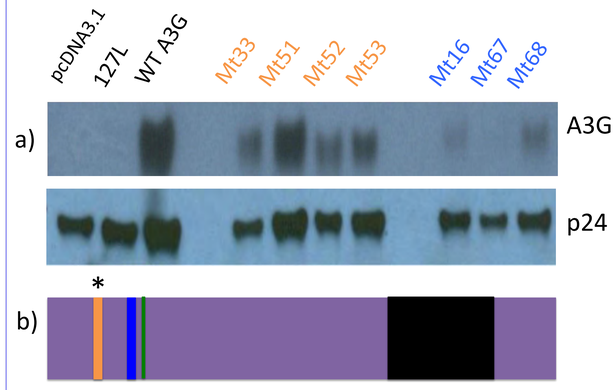


Figure 3. Virion Packaging a) Virus-containing supernatant was purified through a sucrose cushion. The presence of A3G and p24 in isolated virions was detected by Western Blotting **b)** The two identified novel regions reside in the N terminus of the A3G protein whereas the previously-characterized catalytic domain is in the C terminus. Novel domain I is indicated in **orange**, novel domain II is in **blue**, delineated packaging domain is in **green**, and the characterized catalytic domain is in **black**.

CONCLUSIONS

We identified two novel domains within the N terminus of the anti-HIV-1 protein, A3G. Mutation of either of these domains led to a significant decrease in antiviral activity, but did not affect mutagenic properties, suggesting that these two N-terminal domains play a role in A3G's deamination-independent antiviral activity. The expression of A3G was examined and subcellular localization was determined. Mutant proteins that were poorly packaged appeared to form cytoplasmic aggregates. In addition previous work has determined that one of the two regions we have identified is under positive selection, potentially suggesting that this region could be involved in the evolutionary arms race between A3G and HIV-1 Vif.

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