Combating HIV: Exploring the Structure of APOBEC3G (A3G)

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1. A3G Can Restrict HIV

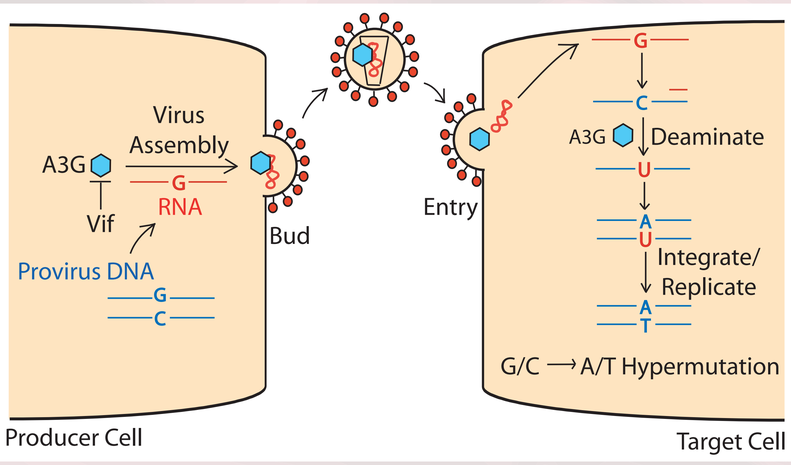


Fig 1. APOBEC3G functions by deaminating retroviral DNA cytosines to uracils (1). The uracils in turn are replicated as thymines and result in high levels of C-to-T hypermutation. These APOBEC-triggered mutations occur so extensively that the virus is rendered non-infectious (2). However, a viral protein, Vif, is capable of inhibiting A3G.

2. What is A3G's Structure?

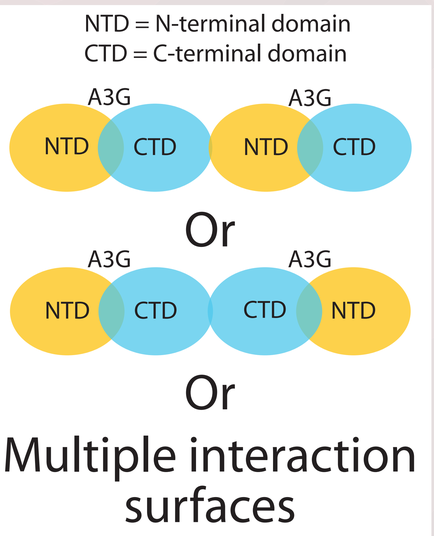


Fig 2. While it is known that A3G interacts with itself, not much more is understood about this self-interaction and whether it is biologically significant. Knowing how A3G polymerizes and whether this polymerizaiton is necessary for activity will improve understanding of A3G's function (3).

3. Identifying Non-Interactors

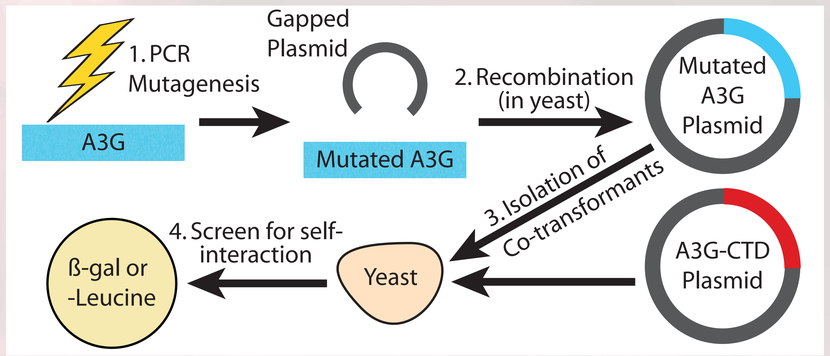


Fig 3. Random mutations were induced in A3G by PCR mutagenesis. Gapped plasmid, the mutated A3G fragmentand a plasmid expressing A3G-CTD were co-transformed into yeast where the gapped plasmid and mutated A3G undergo homologous recombination.

Yeast Two-Hybrid (Y2H) Results

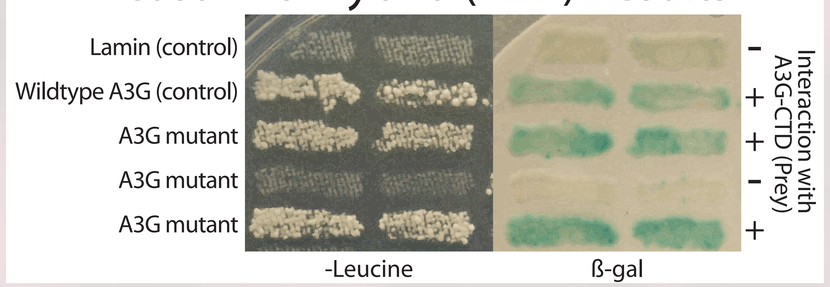


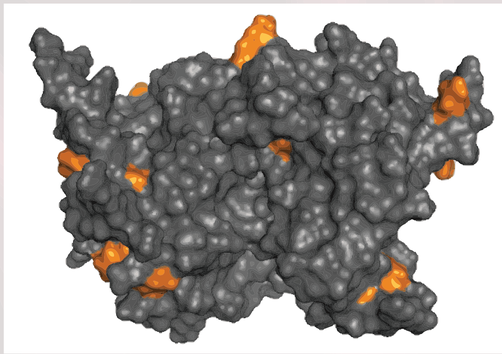
Fig 4. Using a Y2H screen, interactions between the bait and prey plasmids were detected. A positive interaction results in growth on the -leucine plates or blue colonies after a ß-gal assay. We were interested in the mutants that no longer interacted with A3G-CTD, as this would imply a disrupted interaction surface.

4. Elimination of Mutants

- 3,000 mutant yeast colonies were patched.
- 529 patches showed negative (-) interaction in the Y2H.
- 30 of these patches had their (-) interaction confirmed.
- 10 of these mutants had *only* missense mutations.

5. Conclusions

Fig 5. The predicted structure of monomeric A3G. The locations of the mutations are shown in orange.



- The spacial diversity of the mutations may indicate a complex self-interaction.
  - A3G may self-interact over several structural surfaces.
  - A single base replacement may not entirely destroy the self-interaction.
  - This may explain the small number of non-interaction mutants.

6. Future Directions

- Perform western blots to ensure mutant expression.
- Test the mutants' catalytic DNA deaminase activity.
- Perform HIV infectivity assays.
- Determine if A3G's self-interaction is biologically significant.

7. Acknowledgements/References

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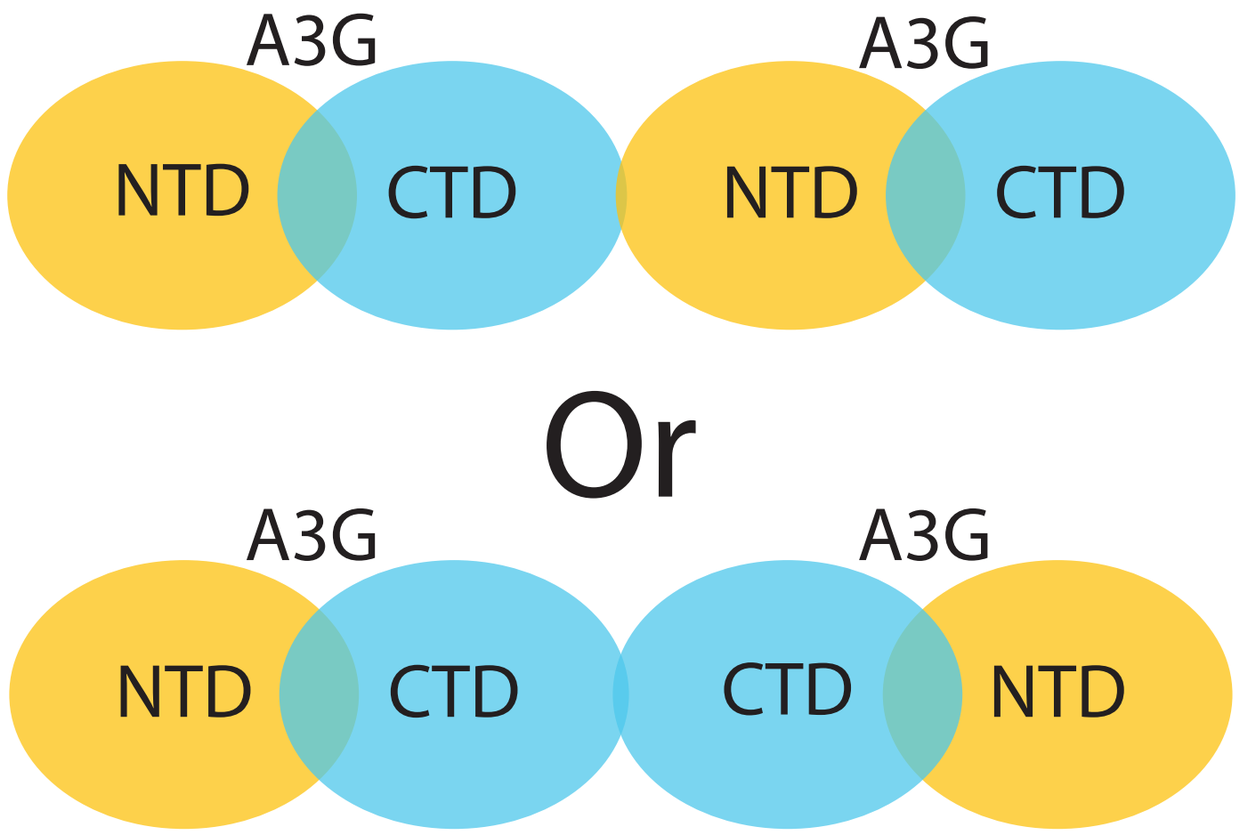
NTD = N-terminal domain
CTD = C-terminal domain



Or

Or

Multiple interaction surfaces

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