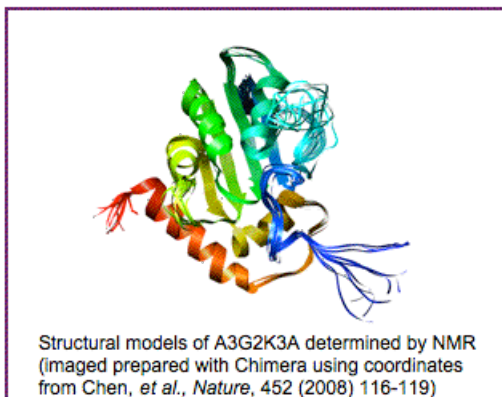


Investigation of Cytidine Deamination in APOBEC3G.

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Background: APOBEC3G, or A3G, is a human protein that has evolved as a defense mechanism against retroviruses. A3G inhibits retroviruses through cytidine deamination of the retroviral genome. We wanted to investigate the specific interactions between A3G and HIV. Goals for our project included synthesizing and purifying the A3G protein, examining its interaction with proteins in HIV, testing the cytidine deamination activity, and evaluating binding between the protein and DNA.

Approach: In order to study A3G, we synthesized the protein in bacteria using a bacterial over-expression plasmid which we constructed by subcloning. In addition to using the full length version, we also used a truncated variant of our protein [A3G2K3A], which had improved solubility. The recombinant proteins were purified by affinity chromatography. After purification, we ran activity and binding assays to examine the interactions between our two versions of A3G and fluorescently labeled DNA.



Results: We have succeeded in synthesizing and purifying the truncated and full length versions of the A3G protein. We observed that both versions could bind to DNA, we observed limited cytidine deamination activity in the full-length version, and no detectable cytidine deamination in the truncated version.

Future Work: For the truncated A3G, we plan to re-sequence the over-expression plasmid. In addition, the sensitivity of the cytidine deamination assay needs to be improved. In the future, we plan to investigate the processivity of A3G on long DNA substrates.

Acknowledgements:

Prof. Karl Haushalter
Jennifer Fukuto '09 (HMC)
Caitlin Olmsted '10 (HMC)
Lillian McCollum and Elaine Guerra
Biology Department
Pomona College
NSF-REU

