POSTER 42

DISSECTING APOBEC3G SUBSTRATE SPECIFICITY BY NUCLEOSIDE ANALOG INTERFERENCE

Jason W. Rausch[1], Linda Chelico[2], Myron F. Goodman[2] and Stuart F. J. Le Grice[1]

[1]Retroviral Replication Laboratory, HIV Drug Resistance Program, NCI-Frederick, Frederick, Maryland; [2]Department of Biological Sciences, Molecular and Computational Section, University of Southern California, Los Angeles, California

The apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminase genes clustered on chromosome 22 encode a set of enzymes including APOBEC1 (A1), APOBEC2 (A2), APOBEC3A-G (A3A-G), and AID. Although each possesses one or more zinc binding motifs conserved among enzymes catalyzing C→U conversion, the functions and substrate specificities of these gene products vary considerably. For example, although two closely related enzymes, A3F and A3G, both restrict HIV-1 infection in strains deficient in virus infectivity factor (*vif*), A3F selectively deaminates cytosine within 5′-TC-3′ dinucleotide motifs in single stranded DNA, while A3G targets the 3’-terminal C within 5′-CCCA-3′ sequences. In the present study, we have used nucleoside analog interference mapping to probe A3G-DNA interactions throughout the enzyme-substrate complex, as well as to determine which DNA structural features determine substrate specificity. Our results indicate that multiple components of nucleosides within the consensus sequence are important for substrate recognition by A3G (with base moieties being most critical), whereas deamination interference by analog substitution outside this region is minimal. Furthermore, exocyclic groups in pyrimidines 1-2 nts 5′ of the target cytosine were shown to dictate substrate recognition by A3G, with chemical composition at ring positions 3 and 4 found to be more important than at ring position 5. Taken together, these results provide insights into how the enzyme selects A3G hotspot motifs for deamination, as well as which approaches might be best suited for forming a stable, catalytically competent cross-linked A3G-DNA complex for future structural studies.