Nature Reviews Microbiology | Published online 12 Oct 2017; doi:10.1038/nrmicro.2017.129

VIRAL INFECTION

Adapt or get zapped

> CG suppression is key for the replication of HIV-1

Many RNA viruses that infect vertebrates are characterized by a low frequency of CG dinucleotides (where a cytosine nucleotide is followed by a guanine nucleotide) in their genomes, mimicking the dinucleotide composition of their vertebrate host genomes. The explanation for this viral feature, also known as CG suppression, has been unclear.

In a recent study, Takata *et al.* show that viral CG suppression is key for the replication of HIV-1 and that the zinc-finger antiviral protein (ZAP) mediates its antiviral activity by specifically binding to RNA sequences that contain CG dinucleotides and targeting them for degradation, thereby inhibiting viral replication.

To identify *cis*-acting RNA elements that are important for the replication of HIV-1, the authors constructed 16 HIV-1 mutants that contained the maximum number of synonymous mutations (that is, mutations that do not change the amino acid sequence) in viral ORFs. They observed that some of these mutants had replication defects and found that these were caused by the cumulative effect of synonymous mutations. Moreover, they found that synonymous mutagenesis had increased the frequency of CG dinucleotides in the genome of HIV-1 to a similar level to that of a random sequence.

In addition, they observed that viral replication was particularly decreased for an *env* mutant (termed L) and thus derived additional L mutants that had either the new CG dinucleotides only (named L_{CG}), other mutations (named L_{OTH}), the maximum possible number of CG dinucleotides (named $L_{CG\text{-}HI}$) or the same number of inverted GC dinucleotides as a control (named $L_{GC\text{-}HI}$). They found that CG suppression was essential for HIV-1 replication, as only mutants with increased CG levels (L, L_{CG} and $L_{CG\text{-}HI}$) showed a slower replication rate in T cells than the wild-type HIV-1 strain.

HIV-1 replication defects in the CG-enriched mutants were associated with a decrease in the production of progeny virions, a reduction in the expression of the viral Env and Gag proteins, and in the levels of unspliced viral RNA. Furthermore, single-molecule fluorescence *in situ* hybridization (smFISH) against Gag RNA, revealed that the reduction of unspliced — and incompletely spliced — RNA was restricted to the cytoplasm, whereas levels of unspliced RNA in the nucleus were unaffected.

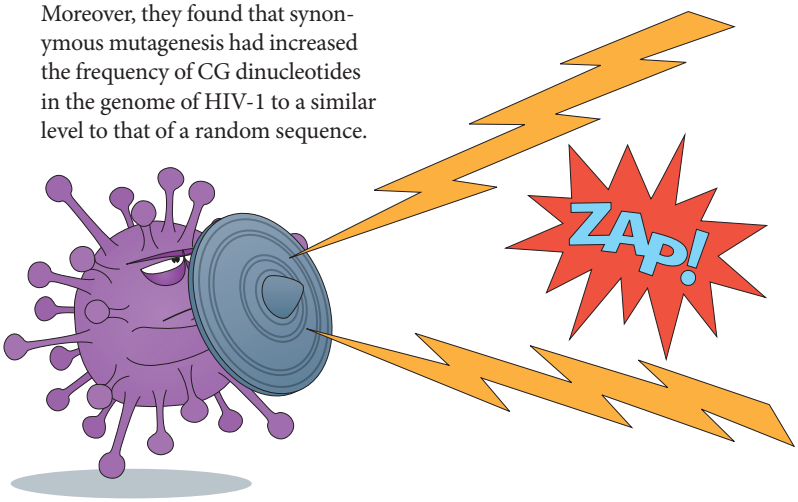
To identify which factor was responsible for the reduction in RNA with high CG dinucleotide content, the authors carried out a focused screen using small inhibitory RNAs (siRNAs) that targeted proteins involved in RNA degradation pathways, and found that ZAP specifically inhibited the replication of CG-enriched HIV-1 mutants. Indeed, by knocking down ZAP they could restore virion yields to almost wild-type levels in cells infected with $L_{CG\text{-}HI}$. Moreover, this mutant showed the same replication dynamics as the wild-type HIV-1 strain in ZAP-deficient cells.

Finally, crosslinking immunoprecipitation coupled with high-throughput sequencing (CLIP-seq) analyses showed that ZAP directly and preferentially binds to regions that contain CG dinucleotides in RNA sequences, and through this mechanism it can distinguish between self and non-self RNA. Indeed, host genomes show marked CG suppression at the level of mRNA ORFs and 3′ untranslated region (UTR) sequences.

In summary, this study suggests that CG suppression in HIV-1 and other RNA viruses of vertebrates could represent an additional immune defence mechanism and that ZAP, by specifically binding to CG-rich RNA sequences, can identify non-self RNAs and target them for degradation.

These findings provide the basis for the manipulation of viral CG content or of ZAP activity that could be used for the development of new expression vectors and live attenuated vaccines.

Irene Vacca



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ORIGINAL ARTICLE Takata, M. A. *et al.* CG dinucleotide suppression enables antiviral defence targeting non-self RNA. *Nature* http://dx.doi.org/ 10.1038/nature24039 (2017)