Vif- H3.3 interaction, a new Vif function?

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INTRODUCTION

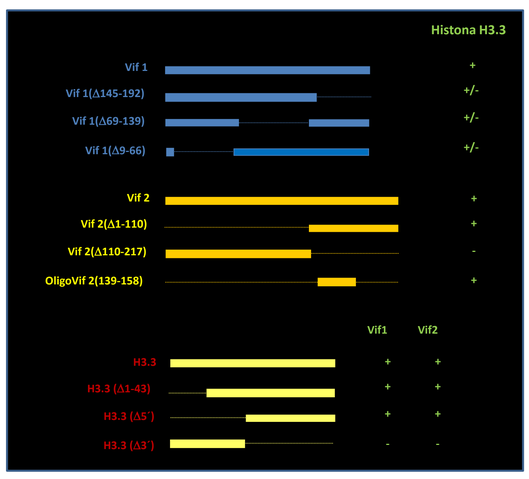
HIV virus encodes for several accessory proteins in particular Vif protein that is essential in an early phase of viral infection.

In 2002, it was demonstrated an interaction between Vif and APOBEC (1), that inhibits APOBEC (cytidine deaminase) function and at the same time reduces APOBEC incorporation in the virion.

Earlier studies suggested that Vif functions in viruses producing cells (permissive and non permissive) but its activity influences early stages of viral infection (2). Other studies also revealed that Vif is necessary to maintain productive viral infection (3).

We have performed several two-hybrid screenings of cDNA libraries (from PBMC, IL-2 stimulated), using Vif1 and Vif2 as baits. In all screenings we have identified a specific interaction with Histone 3 variant, H3.3, whereas interactions with histones H2A and H2B, and with other HIV proteins, used as controls, were negative.

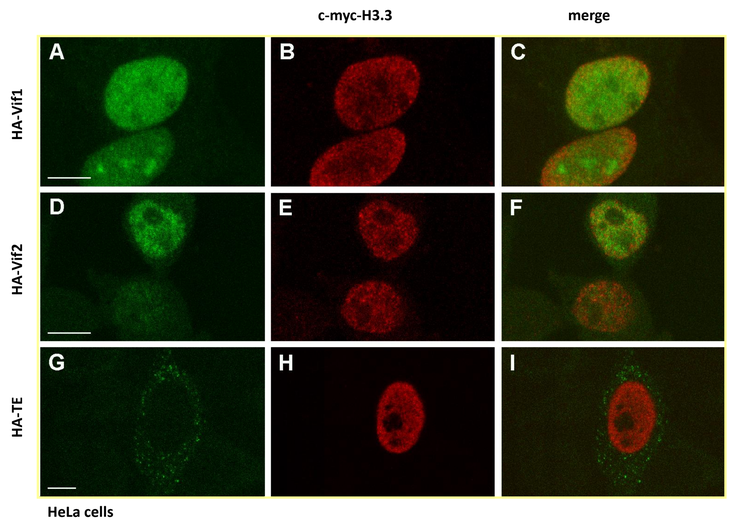
1. Two-hybrid system mapping domains of Vif/H3.3 interaction



Using the two-hybrid system we have identified the interacting domain between Vif2 and histone H3.3.

We used different constructs from H3.3, Vif1 and Vif2, as well as, a conserved motif from Vif2 (named OligoVif2) that is localized between 139 and 158 aa residues. As we can observed the domain is localized in the COOH terminal of H3.3 histone and Vif2 protein. For Vif1 and with the constructs that we have used the results are not so conclusives.

2. Cellular localization of HA-Vif1, HA-Vif2 and cmyc-H3.3 in HeLa transfected cells

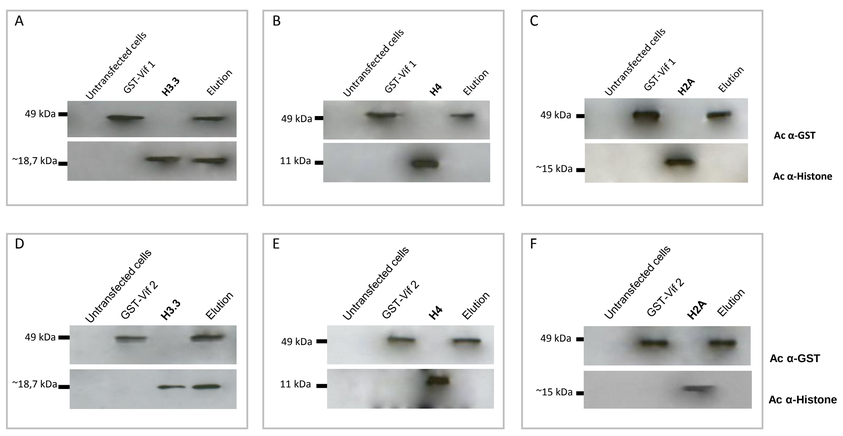


HeLa cells were co-transfected with HA-Vif1, HA-Vif2, HA-TE and c-myc histone H3.3. Immunolocalization was performed at 19h pos-transfection fixeted cells.

HA-TE (a peroximal enzyme), was used has negative control for HA tag.

On C, F, I its possible to see merge of both tag detections. On panel A and D we see that both Vif1 and Vif2 have nuclear localization, same results were obtain with suspension cell lines, like T cell lines (H9) and macrophagic cell lines (U38).

3. GST pull-down assay to study Vif/Histone H3.3 interaction



On panel A was shown GST-Vif/H3.3 interaction with Vif from HIV1, as well as, on panel D but in this case the interaction was between Vif from HIV2.

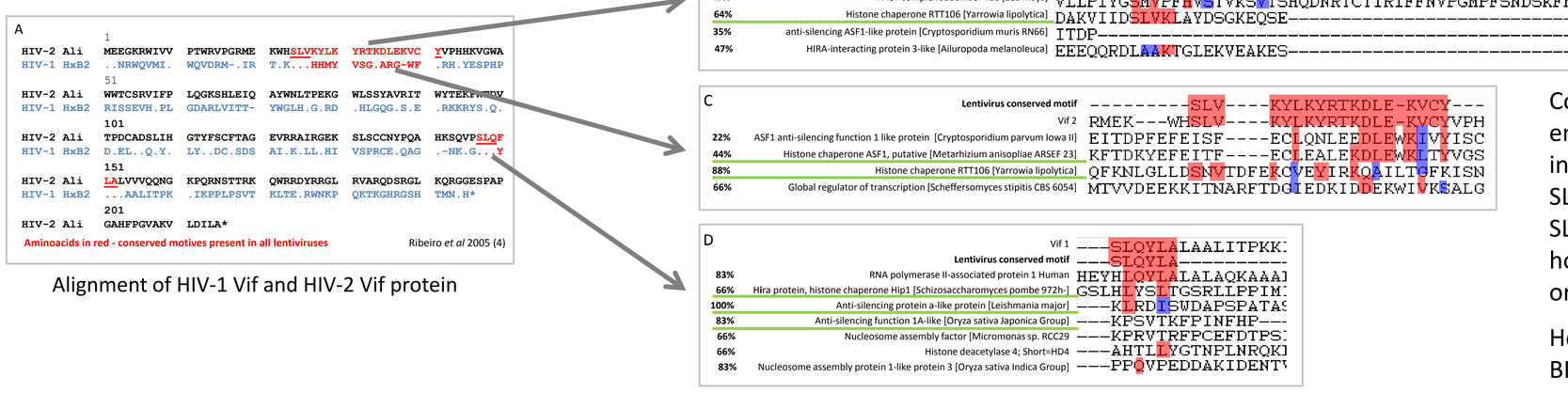
Vif/H4 and H2A interactions were used as negative control (panel B, C, E and F).

GST-Vif (1 and 2) unpurified proteins were incubated with 6 µg of each histone. Proteins were analyzed by Western blot with anti-GST and anti-histone antibodies.

GST pull-down assays have confirm *in vitro* Vif/H3.3 histone interaction.

When we use H2A, H2B and H4 in the same assay, we obtain negative results, suggesting that those histone don't interact with Vif proteins.

4. Homology sequence analysis between conserved amino acid residues from Vif1 and Vif2 proteins and Histone chaperons



Alignment of HIV-1 Vif and HIV-2 Vif protein

Conserved Vif motives, namely the sequence that encodes the amino acid residues – SLQYLA, present in all lentiviruses and other conserved motifs SLVKHHMYVSGKARGWFY (Vif1) and SLVKYLKRTKDLEKVCY (Vif2) have significant homology with ASF1 from several eukaryotic organisms.

Homology sequence analysis was performed using BLAST and COBALT program to align multiple protein sequences.

CONCLUDING REMARKS

Our results show that Vif interacts specifically with H3.3 and also with H3 (data not shown), which only differs in 2 amino acids. The high levels of homology obtained between conserved sequences of Vif1 and Vif2 and histone chaperones indicate that Vif could act as a histone chaperon, specific for histone H3.3 and histone H3.

Although biological relevance of this interaction is still not well defined, if we compare our results with results obtain with others viruses that showed interactions between viral proteins and histone chaperones that result in viral replication activation (5), we hypothesize that Vif acts as a histone chaperon itself, playing an important role in transcriptional activation, since H3.3 is an epigenetic positive tag for activation of transcription, in chromatin modulation. Inactivation of H3.3 gene will clarify the biological meaning of this interaction for HIV replication.

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