J Neuroimmune Pharmacol (2011) 6:296–307
DOI 10.1007/s11481-011-9258-7

ORIGINAL ARTICLE

HIV-1 Vif Interaction with APOBEC3 Deaminases and its Characterization by a New Sensitive Assay

Iris Cadima-Couto · Nuno Saraiva · Ana Catarina C. Santos · Joao Goncalves

Received: 31 October 2010 / Accepted: 12 January 2011 / Published online: 29 January 2011

Abstract The human APOBEC3 (A3) cytidine deaminases, such as APOBEC3G (A3G) and APOBEC3F (A3F), are potent inhibitors of Vif-deficient human immunodeficiency virus type 1 (HIV-1). HIV-1 Vif (viral infectivity factor) binds A3 proteins and targets these proteins for ubiquitination and proteasomal degradation. As such, the therapeutic blockage of Vif–A3 interaction is predicted to stimulate natural antiviral activity by rescuing APOBEC expression and virion packaging. In this study, we describe a successful application of the Protein Fragment Complementation Assay (PCA) based on the enzyme TEM-1 β-lactamase to study Vif–A3 interactions. PCA is based on the interaction between two protein binding partners (e.g., Vif and A3G), which are fused to the two halves of a dissected marker protein (β-lactamase). Binding of the two partners reassembles β-lactamase and hence reconstitutes its activity. To validate our assay, we studied the effect of well-described Vif (DRMR, YRHHY) and A3G (D128K) mutations on the interaction between the two proteins. Additionally, we studied the interaction of human Vif with other members of the A3 family: A3F and APOBEC3C (A3C). Our results demonstrate the applicability of PCA as a simple and reliable technique for the assessment of Vif–A3 interactions. Furthermore, when compared with co-immunoprecipitation assays, PCA appeared to be a more sensitive technique for the quantitative assessment of Vif–A3 interactions. Thus, with our results, we conclude that PCA could be used to quantitatively study specific domains that may be involved in the interaction between Vif and APOBEC proteins.

Keywords HIV-1 · Vif · APOBEC3G · Protein interaction · Drug screening

Abbreviations

PCA	Protein Fragment Complementation Assay
HIV-1	Human immunodeficiency virus type-1
Vif	Viral infectivity factor
A3G	Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G
A3F	Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3F
A3C	Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3C

Introduction

Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (A3G) is a single-stranded DNA cytidine deaminase that targets lentiviral minus-strand DNA and has potent antiviral activity against diverse retroviruses. In order to successfully replicate in their hosts, lentiviruses such as HIV-1 and simian immunodeficiency virus (SIV) encode the Vif protein, which induces polyubiquitination and proteasomal degradation of multiple A3 molecules (Conticello et al. 2003; Liu et al. 2004; Marin et al. 2003; Mehle et al. 2004a, b; Sheehy et al. 2003; Stopak de Noronha et al. 2003; Yu et al. 2003). Together with

Iris Cadima-Couto and Nuno Saraiva contributed equally to the manuscript.

This study received support through FCT grant POCI/BIA-MIC/60038/2004.

I. Cadima-Couto · N. Saraiva · A. C. C. Santos · J. Goncalves (✉)
URIA-IMM, Faculdade de Farmácia da Universidade Lisboa,
Av. Das Forças Armadas,
1649-059 Lisbon, Portugal
e-mail: joao.goncalves@ff.ul.pt

A3G, A3F displays the most potent effect against HIV-1 (Bishop et al. 2004). Other A3 family members, such as A3C, have weaker anti-HIV-1 activities and are only partially degraded by Vif (Marin et al. 2008).

Recent advances in the study of the biological role of HIV-1 Vif and A3 proteins, together with progress in deciphering how Vif counteracts A3G and A3F opened new opportunities to develop anti-HIV drugs. Understanding the mode of action of Vif and A3G can provide a number of attractive targets for drug development since A3G displays the most potent activity against HIV-1. Inhibition of this interplay could allow the host innate defences to control viral replication.

Disruption of Vif–A3G interaction is predicted to rescue A3G expression and virion packaging, consequently stimulating intracellular antiviral activity. Similarly, pharmacologic studies to suppress A3G proteasome-mediated degradation have been shown to enhance A3G half-life and consequently inhibit HIV-1 infection (Douaisi et al. 2005; Yu et al. 2003). In order to facilitate the rational design of inhibitors of Vif–A3G interaction, experimental assays have been devised to define features of Vif that are involved in the interaction with A3G and vice-versa.

In the last decade, many residues have been described as being involved in the interaction between the two proteins. The N-terminal region of HIV-1 Vif is important for binding and neutralization of A3G and A3F and also contributes to species-specific recognition (Marin et al. 2003; Indrani et al. 2006; Simon et al. 2005; Schrofelbauer et al. 2006; Tian et al. 2006). The highly conserved cysteine residues at positions 114 and 133 and the $S^{144}LQXLA^{149}$ motif (Fig. 1a) are required for Vif function and HIV-1 replication (Goncalves et al. 1994; Ma et al. 1994). Vif associates with the Cul5–EloB–EloC complex by binding directly to EloC via a BC box motif at positions 144 to 150 and to Cul5 via hydrophobic residues at positions 120, 123, and 124 within a zinc-binding region formed by the highly conserved HCCH motif (Fig. 1a) (Mehle et al. 2004b, 2006). The SLQXLA motif is essential for targeting A3G for proteasomal degradation. Substitution of the SLQ portion in SLQXLA motif has been reported to be sufficient to prevent A3G degradation (Kobayashi et al. 2005; Sheehy et al. 2003; Yu et al. 2003). The zinc-binding motif HCCH is also involved in A3G degradation and is necessary for the specificity of Vif–Cul5 interaction (Liu et al. 2005; Luo et al. 2005; Xiao et al. 2007). Finally, the conserved proline-rich $P^{161}PLP^{164}$ domain of Vif (Fig. 1a) was shown to be crucial for Vif function and viral infectivity (Simon et al. 1997; Yang et al. 2001, 2003). It can interact with A3G (Donahue et al. 2008; Miller et al. 2007), Cul 5 (Yu et al. 2004a, b), Elo B (Bergeron et al. 2010; Wolfe et al. 2010), and HIV-1 reverse transcriptase (Kataropoulou et al. 2009). In addition, mutations in this domain were shown to eliminate Vif oligomerization and nucleic acid binding properties (Bernacchi et al. 2010). Indeed, Vif has been shown to self-associate and form dimers, trimers, and tetramers in vitro (Auclair et al. 2007; Yang et al. 2001), as well as in mammalian cells (Yang et al. 2001).

Several groups have shown that Vif-induced degradation of A3G requires the physical interaction of the two proteins and that a single amino acid change in A3G at residue 128 is sufficient to abolish this interaction (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004). This assumption led to the conclusion that Vif–A3G interaction is species-specific and is determined by aspartic acid at position 128 in A3G and lysine in African Green Monkey (AGM; Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004; Xu et al. 2004). Substitution of lysine 128 in human A3G (D128) with aspartic acid in AGM A3G, results in a mutant (D128K–A3G) protein that is resistant to Vif. This can be, either because the mutant protein is no longer able to interact with Vif or due to inhibition of subsequent downstream effects (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2006; Xu et al. 2004).

Asparagine at position 128 in A3G was shown to interact with amino acids 15 or 17 of Vif, and mutations in the DRMR conserved region of Vif can also alter its species-specificity (Schrofelbauer et al. 2006). Substitution of the DRMR sequence at positions 14 to 17 to SERQ or SEMQ, which resembles the SIV Vif sequence, allowed Vif to counteract the antiviral activity of AGM A3G and Rh A3G (Schrofelbauer et al. 2006). The loss of species restriction is probably caused by an overall increase in the negative charge of amino acids in the 14–17 region of HIV-1 Vif, which promotes effective interaction with the positive charge of lysine present at residue 128 in AGM A3G and Rh A3G. In addition, the DRMR region was also shown to be critical for the strength of binding with A3G (Schrofelbauer et al. 2006), but additional binding sites may be required for stabilization of this interaction (Fig. 1a; Russell and Pathak 2007).

By performing an extensive mutational analysis of Vif, Russel, and Pathak identified a new motif, $Y^{40}RHHY^{44}$ motif, which was shown to be involved in binding to A3G (amino acids 126–132; Fig. 1a; Russell and Pathak 2007). Vif $Y^{40}RHHY^{44}$ motif was considered a critical domain for binding to A3G while the $D^{14}RMR^{17}$ domain could be involved in a secondary step involving A3G degradation (Russell and Pathak 2007).

In vitro binding assays and cell-based assays have been commonly used to identify binding regions between Vif and A3G. However, these approaches offer some disadvantages as the in vitro processing steps make it difficult to assess the significance of the result. Probably due to improper folding, cell-based assays are often complicated due to the relative instability or structural dislocation of Vif

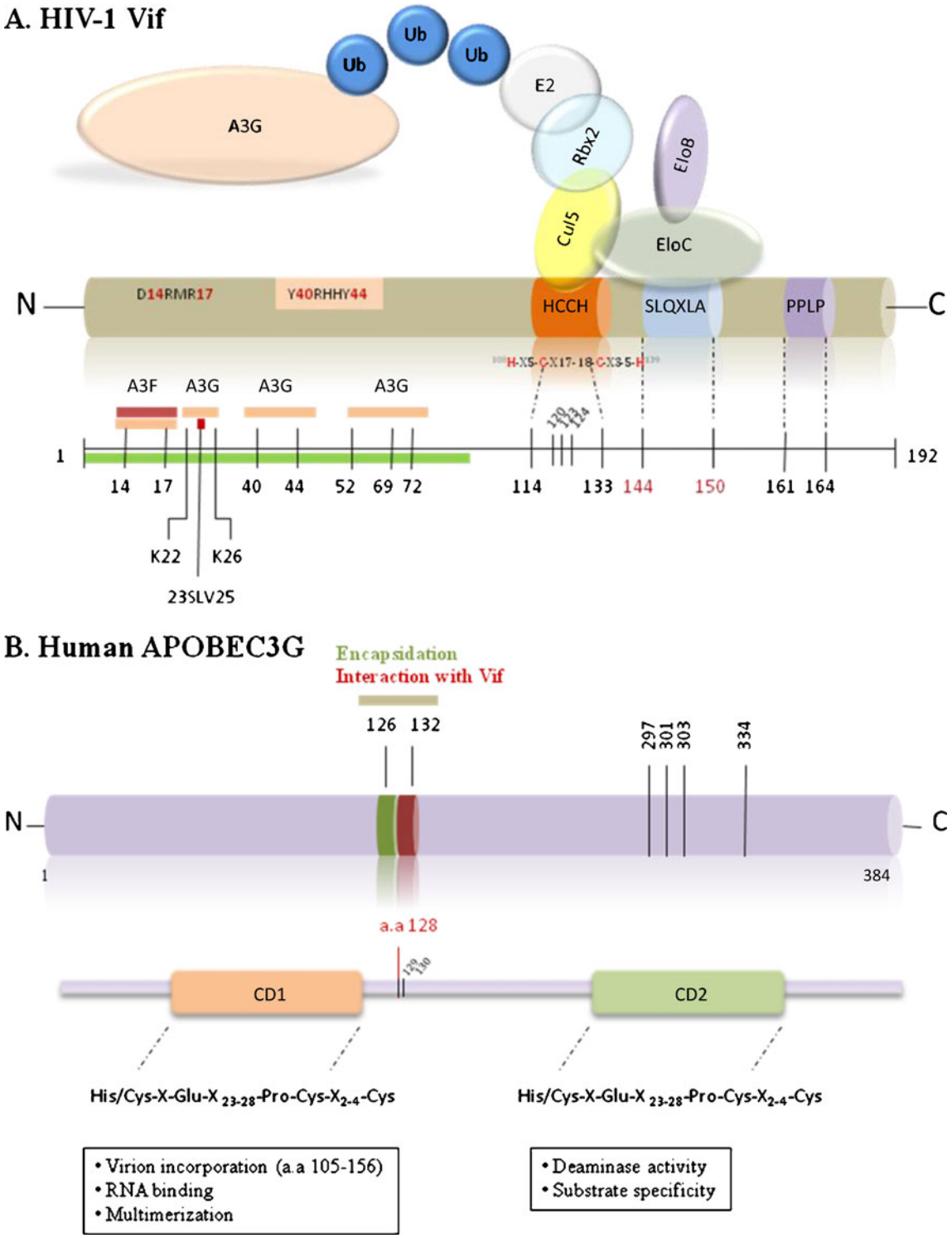


Fig. 1 Schematic representation of Vif and A3G domains involved in the interaction of both proteins. **a** Vif binds to A3G through specific residues located in the N-terminal region. Amino acids in Vif that are involved in the interaction with A3G are shown in *pink*. Vif C-terminal domains, involved in targeting A3G for proteasomal degradation, are shown in *orange* (zinc-binding HCCH domain), and *light blue* (SLQXLA). The multimerization domain is *purple*. **b** The catalytic domains (CD1 and CD2) and Vif-binding regions of A3G protein are represented. Amino acids 126–132 are involved in A3G virion incorporation and interaction with Vif and are represented in *green* and *red*

deletion mutants (Marin et al. 2003). In order to circumvent some of these problems, we used a strategy for detecting Vif and APOBEC interactions based on protein interaction-assisted folding of rationally designed fragments of enzymes. This strategy is called Protein Fragment Complementation Assay (PCA). This system circumvents some of the problems of co-immunoprecipitation such as: (a) inefficient washing of non-bound proteins from the solid support and beads loss due to decanting; (b) antibody fragment interference; (c) harsh elution conditions; (d) type of antibody that can significantly affect the outcome of binding interaction. Moreover, co-immunoprecipitation requires a great deal of optimization and troubleshooting in high-throughput setting.

PCA has been used for many purposes, including the study of protein folding (Ladurner et al. 1997; de Prat et al. 1994), gene structure (Ullmann et al. 1967), the role of primary sequence in determining tertiary structure of proteins (Taniuchi and Anfinsen 1971), the role of tertiary structure elements in enzyme catalysis (Shiba and Schimmel 1992), to probe macromolecular assembly (Tasayco and Carey 1992), and to test theories on protein evolution (Bertolaet and Knowles 1995). More recently, Pelletier and others (de Virgilio et al. 2004; Pelletier et al. 1998; Philippon et al. 1998; Remy et al. 2004) have used the PCA approach for the in vivo detection of protein–protein interactions. PCA relies on engineering reporter protein fragments that exhibit no functional activity by themselves and do not spontaneously fold. When these fragments are fused to interacting proteins, binding will bring the two reporter fragments into proximity. Fragments will then fold into an active three-dimensional (3D) structure of the complete reporter protein (Remy and Michnick 1999; Remy et al. 1999). Several proteins have been described as reporter proteins in PCA such as β-lactamase, dihydrofolate reductase, *Renilla*, luciferases, green fluorescent protein, and yellow fluorescent protein (Galarneau et al. 2002; Spotts et al. 2002; Remy and Michnick 2001; Paulmurugan et al. 2002; Luker et al. 2004; Ghosh et al. 2000; Hu et al. 2002; Remy et al. 2004; Subramaniam et al. 2001).

In this study, we used the enzyme TEM-1 β-lactamase (EC, 3.5.2.6) as a reporter protein to detect Vif–A3 interaction. This strategy offered us the advantage of performing the assays in a cell-based system without the requirement for protein co-immunoprecipitation.

Materials and methods

Plasmids

pcDNA3.1-A3G-HA was used for the expression of human A3G protein tagged at C terminus was kindly supplied by Klaus Strebel (Kao et al. 2003). pcDNA-HVif was obtained from the NIH AIDS Research and Reference Reagent Program (Kao et al. 2003). pcDNA3.1 Zeo(+) was obtained from the NIH AIDS Research and Reference Reagent Program (#10077). Plasmids pcDNA3.1-ZIP-15a.a-BLF[1] (ZIP-B1) and pcDNA3.1-ZIP-15a.a-BLF[2] (ZIP-B2) were kindly supplied by W. Michnick and have been previously described (Galarneau et al. 2002).

DNA constructs

Plasmids pcDNA3.1-ZIP-15a.a-BLF[1] (ZIP-B1) and pcDNA3.1-ZIP-15a.a-BLF[2] (ZIP-B2) were used as cloning vectors for the subsequent steps. Each cloning vector was linearized with restriction enzymes NotI/AscI to remove ZIP sequence. For the insertion of HVif, A3G-HA, A3F-HA, A3C-HA, in fusion with β-lactamase fragment, each sequence was amplified by PCR using specific oligonucleotides (HVif: Not-HVif-F and Asc-HVif-R; A3G-HA: A3G-amp-F and 4BL-B1-Asc-R; A3F-HA: F-Not and R-Asc; A3C-HA: A3C-F-Not and A3C-R-Asc). PCR products were digested with NotI/AscI restriction enzymes and ligated to the linear vector. This cloning strategy originated the following plasmids: Vif-B2, A3G-B1, A3G-B2, A3F-B1, and A3C-B1.

Mutation of SLQ region in pcDNA–HVif was accomplished by PCR overlap method of pcDNA–HVif originating the Vif SLQ > AAA mutant. Vif SLQ > AAA mutant was generated using oligos: SLQ-Vif-NIH-F and SLQ-Vif-NIH-R. Substitution of Vif YRHHY and DRMR regions by alanines was accomplished by PCR overlap of Vif SLQ > AAA using specific oligonucleotides (Vif YRHHY: Vif-YRHHY-F and Vif-YRHHY-R; Vif DRMR: Vif-DRMR-F and Vif-DRMR-R).

A3G mutant D128K was generated by PCR using specific oligonucleotides (A3G-D128K-F and A3G-D128K-R). All Vif and A3G mutants generated by PCR were digested with restriction enzymes NotI/AscI and ligated to the linear vector in fusion with β-lactamase fragment. This cloning originated the plasmids: VifSLQ>A3-B2, VifSLQ>A3+YRHHY>A5-B2, VifSLQ>A3+DRMR>A4-B2, and A3GD128K-B1. All constructs were subjected to sequencing analysis for the detection of undesirable mutations. The sequence of primers used is listed in Table 1.

Antisera

Anti-HA Horseradish Peroxidase (HRP) monoclonal antibody (Roche) was used for the detection of A3G HA. Anti-HA High Affinity Matrix (Roche) was used for co-immunoprecipitation assays. Vif was detected using the anti-Vif rabbit polyclonal antibody supplied by the NIH AIDS Research and Reference Reagent Program Catalogue (#2221). GAPDH was used as a loading control, and the antibody used was purchased from Santa-Cruz Biotechnology (GAPDH 6C5). Anti-rabbit IgG HRP antibody was purchased from Cell Signaling (#7074).

Table 1 The sequence of oligonucleotides used for construction of chimeric and mutant proteins

Oligonucleotide	Sequence (5′–3′)
A3G-amp-F	ata aga atg cgg ccg cta aac tat atg aag cct cac ttc aga aac aca gtc
4BL-B1-Asc-R	acc gcc acc ggc gcg cca aga agc gta gtc cgg aac gtc
F-Not	ata aga atg cgg ccg cta aac tat atg tac ccg tac gac gtt ccg gac tac gct tct gcc cag aag gaa gag gct gc
R-Asc	acc gcc acc ggc gcg cca ctc gag aat ctc ctg cag ctt g
A3C-F-Not	ata aga atg cgg ccg cta aac tat atg tac ccg tac gac gtt ccg gac tac gct tct aat cca cag atc aga aac ccg atg
A3C-R-Asc	acc gcc acc ggc gcg cca ctg gag act ctc ccg tag cct tc
Not-HVif-F	ata aga atg cgg ccg cta aaa tgg aga acc ggt ggc agg
Asc-HVif-R	acc gcc acc ggc gcg ccc gtg tcc att cat tga atg gct ccc
A3G-D128K-F	cta ctt ctg gaa gcc aga tta cca g
A3G-D128K-R	ctg gta atc tgg ctt cca gaa gta g
SLQ-Vif-NIH-R	ctg cta gtg cca agt atg ctg ctg ctc ct acct tgt tat gtc ctg ctt
SLQ-Vif-NIH-F	gca gca tac ttg gca cta agc agc att taa taa aa
Vif-YRHHY-F	gca gct gca gct gct gag agc acc aac ccc aag att ag
Vif-YRHHY-R	cg caa agc taa gga ctg gtt cgc agc gct gca gca gct gct gag agc
Vif-DRMR-F	gca gct gca gct att aac acc tgg aag cgc ctg
Vif-DRMR-R	cca ggt gtt aat agc tgc agc tgc cac ctg cca cac aat cat cac c

Cell culture and transfections

Human embryonic kidney cell line 293T was obtained from the NIH AIDS Research and Reference Reagent Program Catalogue and cultivated in Dulbecco's Modified Eagle's medium containing 10% fetal calf serum, 1× penicillin–streptomycin–fungizone mixture and L-glutamine (Bio Whittaker). For transfections, 293T cells were grown to near 70% confluence in 60-mm plates. Twenty micrograms of each plasmid, Vif-B2, Vif SLQ>A3-B2, VifSLQ>A3+YRHHY>A5-B2, VifSLQ>A3+DRMR>A4-B2, and 10 μg of each A3G-B1, A3G-B2, A3GD128K-B1, A3F-B1, A3C-B1, ZIP-B1, and ZIP-B2 were transfected in 60-mm plates, unless otherwise stated. Transfections were carried using the calcium phosphate precipitation method. Total amount of transfected DNA were kept constant in all samples of any given experiment by adding empty pcDNA3.1 plasmid as appropriate. Cells were harvested at 40–44 h post-transfection.

β-lactamase PCA colorimetric assay

Forty to 44 h after transfection, media was removed, and cells were washed twice in phosphate-buffered saline. We resuspended 1/3 of the transfected 293T cells were in 100 μl of 100 mM phosphate buffer (K2HPO4 50 mM, pH=7.0) and lysed them by three freeze–thaw cycles. Cell debris was removed by centrifugation at 16,000×*g* for 2 min at 4°C. To test for β-lactamase activity, 100 μl of phosphate buffer (100 mM, pH=7.0) was added to each sample to a final concentration of 60 mM and containing 2 μl of 10 mM of CENTA β-lactamase substrate (final concentration, 100 μM; Calbiochem).

Western blot and co-immunoprecipitation analysis

The remaining pool of transfected 293T cells was collected for protein detection and co-immunoprecipitation of Vif and APOBEC proteins. Cells were disrupted in 500 μl of lysis buffer (50 mM Tris, pH 7.4, 50 mM NaCl, 1 mM NaCl, and 1% Triton X-100) containing complete protease inhibitor cocktail (Roche).

For co-immunoprecipitation analysis of A3 proteins and Vif, cell extracts were clarified at 13,000×*g* for 3 min, and the supernatant was incubated overnight on a rotating wheel at 4°C with Anti-HA High Affinity Matrix (ROCHE). Immune complexes were washed three times with 50 mM Tris, pH 7.4, 50 mM NaCl, 1 mM NaCl, and 1% Triton X-100 pH 7.4. Bound proteins were eluted from beads by heating in sample buffer for 5 min at 95°C to be analyzed by immunoblotting.

Results

Detection of HIV-1 Vif and human A3G interactions using PCA

The method used in this study involves a simple colorimetric in vitro assay using a chromogenic β-lactamase substrate (CENTA). TEM-1 β-lactamase is a relatively small monomeric protein that has been well characterized

functionally and structurally (Matagne et al. 1998; Remy and Michnick 2001). It has been shown to be easily expressed, and it is not toxic to prokaryotic and eukaryotic cells (Remy and Michnick 2001, 2004). Furthermore, PCA based on β-lactamase can be used in eukaryotes and many prokaryotes without any detectable background activity (Galarneau et al. 2002). A schematic representation of the PCA principle is shown in Fig. 2. In order to test PCA as a valid tool for study of Vif–A3G interaction, we replaced the homodimerizing GCN4 parallel coiled-coil leucine Zipper (ZIP) sequence by Vif and A3G sequences (Fig. 3a) and expressed both plasmids into 293T cells. Leucine zippers (ZIP) are strong interacting proteins that display a strong β-lactamase activity when fused to the two halves of β-lactamase (B1 and B2; Fig. 3d). Therefore, we decided to use ZIP proteins as a positive control in all our subsequent experiments.

Intracellular interaction between Vif and A3G will bring together the two fragments of the β-lactamase (referred here as B1 and B2) allowing the reconstitution of the reporter β-lactamase enzymatic activity. Therefore, by using the PCA principle we aimed to characterize several specific domains that have been previously shown to be involved in Vif-A3G interaction. As seen in Fig. 3b (lane 1), detection of A3G was not possible in the presence of Vif as it triggers its degradation in the proteasome and consequently β-lactamase activity could not be detected. As such, we decided to replace the SLQ region of Vif by alanines. This will render the protein the inability to form the Cul5–EloB–EloC complex, which is responsible for targeting these proteins to the ubiquitin–proteasome pathway and consequent degradation (Conticello et al. 2003; Liu et al. 2004; Marin et al. 2003; Mehle et al. 2004a, b; Sheehy et al. 2003; Stopak de Noronha et al. 2003; Yu et al. 2003, 2004a, b). The replacement of Vif SLQ region by alanines does not affect the ability of protein binding to A3G (Marin et al. 2003) allowing the study of Vif–A3G interaction by PCA. Furthermore, by avoiding proteasomal degradation, we were able to increase the intracellular concentration of both proteins enhancing the detection of β-lactamase activity (Fig. 3b). The sensitivity of PCA to protein concentration could be further confirmed by increasing the intracellular concentration of either Vif SLQ>A3 or A3G proteins, which resulted in an increase in CENTA hydrolysis rate (Fig. 3c). Therefore, in all our subsequent experiments, we decided to use Vif SLQ>A3 mutant instead of Vif WT (Figs. 4 and 5).

Fig. 3 Detection of HIV-1 Vif and human A3G interaction using the PCA principle. **a** Schematic representation of Vif, Vif SLQ>A3, and A3G fusions to the β-lactamase fragments B2 and B1, respectively. **b** *Upper panel*: hydrolysis rate of CENTA substrate for Vif, Vif SLQ>A3 and A3G interacting pairs of proteins. Hydrolysis of CENTA for fusion proteins alone is also represented as negative control; *Lower panel*: Western blot analysis of cell lysates and co-immunoprecipitation. **c** *Upper panel*: Hydrolysis of CENTA after transfection of increasing amounts of A3G or Vif plasmid DNA. Briefly, 10 μg of A3G-B1 along with increasing amounts of Vif SLQ > AAA-B2 (0, 5, 10, and 20 μg) or 20 μg of Vif SLQ > AAA-B2 along with increasing amounts of A3G-B1 (0, 3, 5, and 10 μg) were used; *Lower panel*: Western blotting analysis of cell lysates and co-immunoprecipitation. **d** *Upper panel*: Hydrolysis of CENTA for interacting pairs of homodimerizing GCN4 leucine zipper (ZIP) plasmids. Briefly, 10 μg of each ZIP plasmid (ZIP-B1 and ZIP-B2) was used. DNA amounts were adjusted to 30 μg using empty pcDNA3.1 vector DNA, when appropriated. Mean hydrolysis rate was determined from four independent experiments to normalized for cell number and protein content

Detection of A3G protein dimerization by PCA

A3G is known to form dimers trough interactions within its C terminus (Bennett et al. 2008). In order to detect intracellular A3G dimerization, we expressed A3G proteins in fusion with the two halves of the β-lactamase enzyme (A3G-B1 and A3G-B2). As similar to positive control (ZIP-B1 and ZIP-B2), hydrolysis of CENTA substrate from 293T lysates transfected with equal amounts of A3G-B1 to A3G-B2 showed a strong interaction [Fig. 3b (lane 7)]. Interestingly, when we added RNase A to the lysates, β-lactamase activity was not inhibited (data not shown) suggesting an RNA-independent oligomerization of A3G, which is in accor-

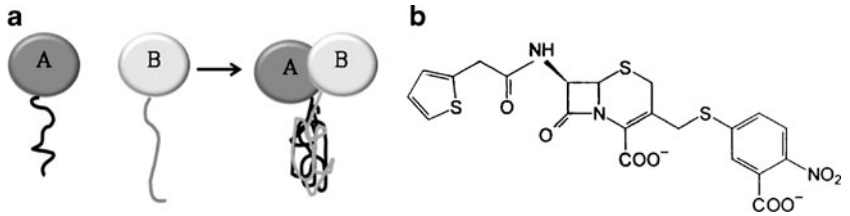
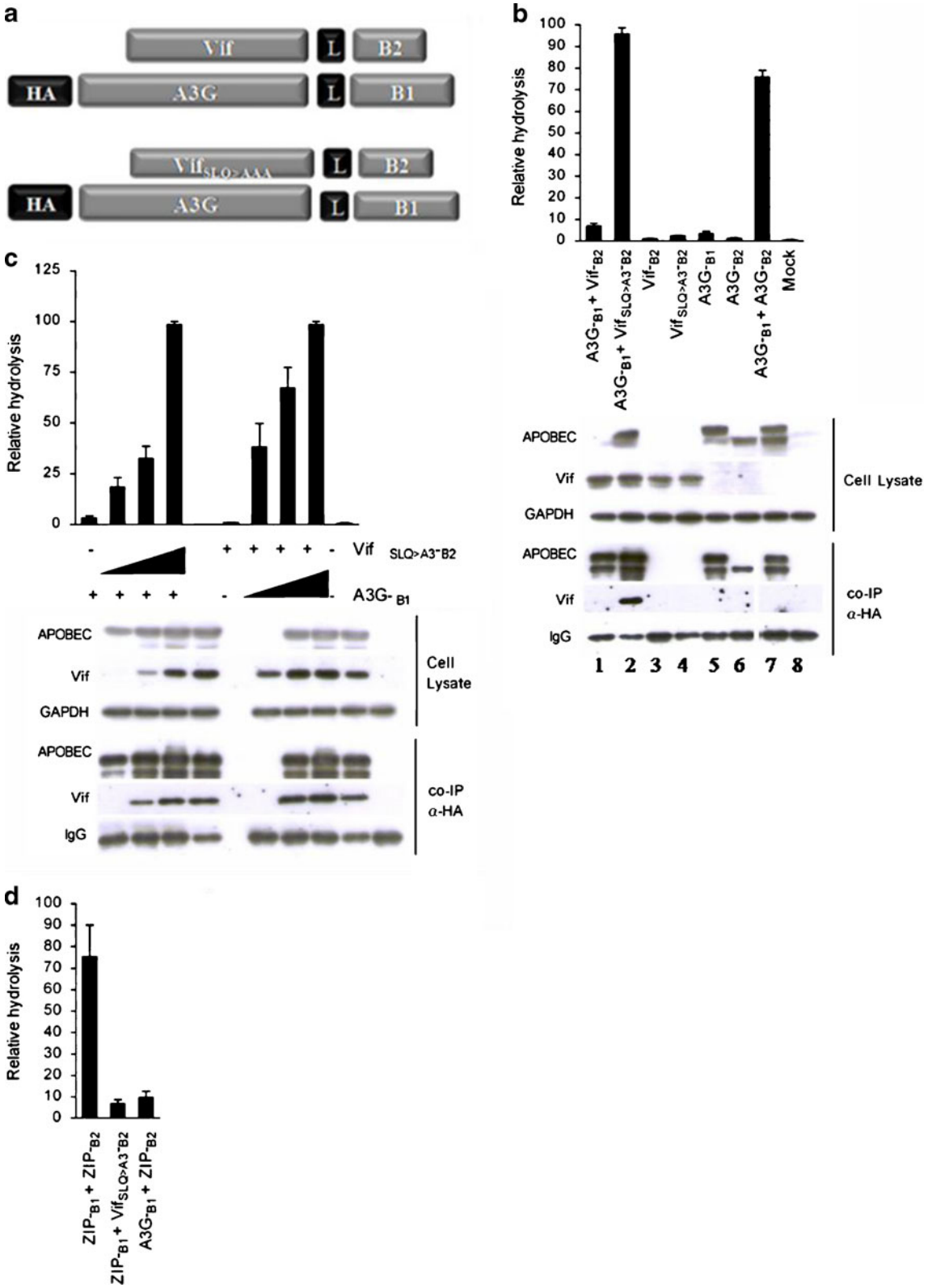


Fig. 2 Schematic representation of PCA strategy used to study protein–protein interaction. **a** Intracellular interaction between proteins *A* and *B*, fused to fragments of β-lactamase, bring fragments into proximity allowing correct folding and reconstitution of enzyme activity. **b** The in vitro assay uses a chromogenic cephalosporin (CENTA), which is readily hydrolysed by β-lactamases. Hydrolysis of the beta-lactamase ring causes a color change from *light yellow* (λmax, 340 nm) to *chrome yellow* (λmax, 405 nm; figure from: Bebrone et al. 2001



dance with a previous report (Bennett et al. 2008). Finally, all interactions were specific as observed in control experiments using non-interacting proteins (e.g., A3G-B1 and ZIP-B2).

Analysis of Vif and A3G mutations by PCA

One of the key residues of A3G that have been extensively studied is amino acid 128 in A3G that is responsible for the

species-specific recognition of A3G by Vif (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004; Xu et al. 2004). Replacement of aspartic acid, which naturally occurs at position D128 in human A3G, with lysine present in AGM A3G, abrogates the interaction with HIV-1 Vif. However, it is still unclear whether A3G D128K is no longer able to interact with human Vif (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004) or, following Vif-A3G D128K binding, a subsequent downstream step is inhibited (Xu et al. 2004). Nonetheless, this residue (D128) is a good candidate to prove the applicability of the β-lactamase assay, since it has been shown to be pivotal in the formation of Vif-A3G complexes (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004; Xu et al. 2004).

According to our results, A3G D128K mutation greatly impaired interaction with human Vif (Fig. 4a) supporting previous reports of Vif–A3G interaction (Bogerd et al. 2004; Mangeat et al. 2004; Schrofelbauer et al. 2004). This was further confirmed by co-immunoprecipitation analysis, in which the D128K mutation reduced the binding Vif to A3G (Fig. 4a, lower panel).

The DRMR and YRHHY regions of Vif have been implicated in the interaction with A3F and A3G, respectively (Russell and Pathak 2007). Therefore, to validate our assay, we replaced these amino acids by alanines and tested their effect on the interaction with A3G and A3F. Our results using PCA showed that Vif YRHHY>A5 mutation impaired the interaction with A3G and A3F by nearly 90% (Fig. 4b, lane 2) and 40% (Fig. 4c, lane 2), respectively. This result underlines the importance of this region for Vif–A3G interaction. When we replaced Vif DRMR region by alanines, we observed that despite having a stronger effect on A3F interaction (80% reduction), it also affected interaction with A3G by more than 50% (Fig. 4b, lane 3). This result is in accordance with a previous report showing that Vif DRMR sequence may be critical for the binding affinity with A3G, although it may not be the sole determinant of this protein–protein interaction (Schrofelbauer et al. 2006). Surprisingly, co-immunoprecipitation analysis showed that Vif DRMR mutation did not affect its ability to interact with A3G (Fig. 4b, lower panel, lane 3). This data indicates that co-immunoprecipitation assays may not be as sensitive as PCA for a quantitative assessment of protein–protein interactions and can sometimes lead to misinterpretation of the results.

Analysis of Vif interaction with A3F and A3C by PCA

A3F has strong antiviral activity, however, its effect on viral restriction is less pronounced than A3G (Holmes et al. 2007; Zennou and Bieniasz 2006). Additionally, A3C has been reported to be relatively resistant to Vif action (Langlois et al. 2005; Yu et al. 2004a, b). According to this, we decided to test the ability of HIV-1 Vif to interact with A3F and A3C. As observed in Fig. 4, Vif–A3C interaction displayed the lowest β-lactamase activity (23%). It has been reported that A3C is partially degraded by Vif

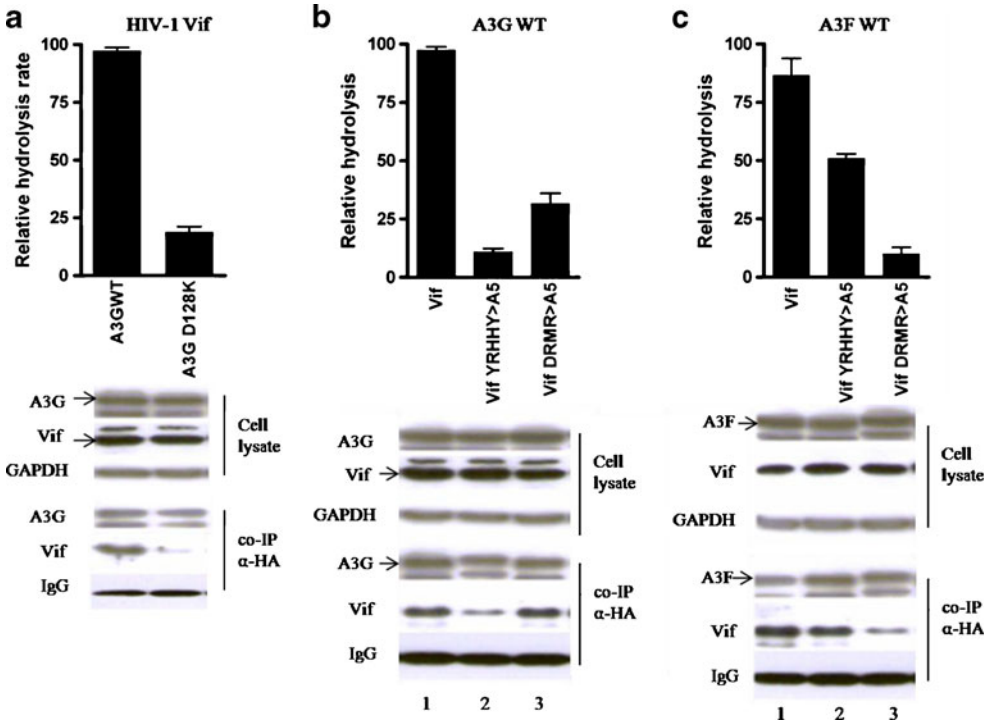


Fig. 4 Effect of A3G and Vif mutations on protein–protein interaction. *Upper panels*: Relative hydrolysis rate of CENTA determined from lysates of 293T cells transiently transfected with: **a** Vif SLQ > AAA-B2 together with A3G WT-B1, or A3G D128K-B1; **b** A3G WT-B1 together with either Vif SLQ > AAA-B2, Vif SLQ > AAA YRHHY>A5-B2, or Vif DRMR>A4-B2; **c** A3F WT-B1 with either Vif WT-B2, Vif YRHHY>A5-B2, or Vif DRMR>A4-B2. *Lower panels*: Western blotting analysis of cell lysates and co-immunoprecipitation. Mean hydrolysis rates were determined from four independent experiments to normalized for cell number and protein content

(Marin et al. 2008), suggesting an unstable or weak interaction which is in accordance with our PCA results. Surprisingly, co-immunoprecipitation analysis demonstrated a similar binding Vif to both A3F and A3C (Fig. 5, lanes 2 and 3). Again, our results indicate that co-immunoprecipitation assays may not be as sensitive and specific as PCA for the discrimination between stable/strong and unstable/weak protein–protein interactions.

Conclusions

A detailed knowledge of the protein domains involved in Vif–A3G interaction is extremely important for the rational design of new antiviral drugs. Antiviral drugs that could inhibit Vif and enhance A3G/A3F activity are emerging as attractive candidates. Until this date, several regions in Vif and A3G have been mapped, and the effect on their interaction was studied. Nevertheless, the three-dimensional molecular structure of both proteins has not yet been determined, and only theoretical predictions providing structural information on Vif domains are available (Auclair et al. 2007; Balaji et al. 2006; Barraud et al. 2008; Huthoff and Malim 2007; Lv et al. 2007; Zhang et al. 2008).

Blocking the binding of Vif to A3G in vivo is certainly one of the most obvious therapeutic strategies. Several authors reported that Vif may function at multiple levels to prevent incorporation of A3G into viral particles (Mehle et al. 2004a; Kao et al. 2007; Santa-Marta et al. 2005; Stopak de Noronha et al. 2003). Therefore, inhibiting the binding of Vif to A3G may have two outcomes: (1) reduction in A3G proteasomal degradation and (2) increase the amount of A3G at viral assembly locations, resulting in higher level of A3G incorporation into virions.

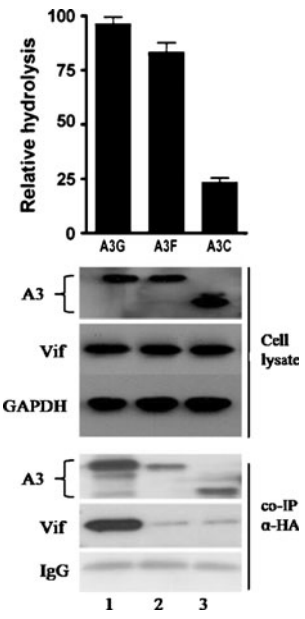


Fig. 5 Vif interaction with A3F and A3C. *Upper panel*: Relative hydrolysis rate of CENTA determined from lysates of 293T cells transiently transfected with Vif-B2 and either A3G-B1, A3F-B1, or A3C-B1. *Lower panel*: Western blotting analysis of cell lysates and co-immunoprecipitation. Mean hydrolysis rates were determined from four independent experiments to normalized for cell number and protein content

As described in this study, the most important domain in A3G responsible for Vif interaction involves the asparagine at position 128 and its surrounding residues. The charge of A3G D128 amino acid markedly influences the interaction with Vif indicating that the contact is dependent on electrostatic forces (Schrofelbauer et al. 2006). Thus, chemical compounds targeting this region could be effective in preventing Vif–A3G interaction. Importantly, molecules targeting amino acid 128 are not predicted to interfere with A3G enzymatic activity, which is conferred by other domains of the protein (Li et al. 2004; Navarro et al. 2005). The screening of unknown target libraries is based on the competition between the bait molecule and unknown target molecules, and high-throughput screening (HTS) assays based on this strategy are often used for discovering inhibitors of protein interaction. The present study demonstrates that β-lactamase complementation is a useful tool that can be applied to HTS assays.

Developing specific and effective small chemical inhibitors to directly inhibit Vif–A3G interaction faces many challenges due to the multiple binding regions involved. In addition, in vitro binding assays and cell-based assays, which have been used to decipher the dynamic principles behind protein functional association, make it sometimes difficult to assess the in vivo significance of the results. In particular, co-immunoprecipitation assays which have been commonly used to study specific domains involved in Vif–A3G interaction are questioned. One study has shown that Vif was able to inhibit virion incorporation and the antiviral activity of an A3G degradation-resistant mutant (C97A; Opi et al. 2007), suggesting a direct inhibition of A3G by Vif. However, the authors could not rule out the possibility that Vif–A3G complexes could have been formed after cell lysis, during co-immunoprecipitation assay. In addition, no one so far has been able to demonstrate a direct Vif–A3G interaction with purified components at physiological concentrations. Therefore, we cannot rule out the need for post-translational modifications, interactions during synthesis, or the need for additional components in the interaction complex.

Moreover, many properties of Vif may influence its interaction with A3G, such as oligomerization, which has been shown to be essential for its function and viral infectivity (Bernacchi et al. 2010). Therefore, it is important to consider that mutations in some Vif domains (e.g., PPLP motif) may induce substantial changes in Vif structure resulting in loss of protein specificity and affinity.

Therefore, assessing the intracellular behavior of Vif and A3G without the requirement for in vitro manipulation is of great importance. This is particularly important in the case

of a multi-functional protein like Vif where the lack of a well-defined structure poses many challenges for its study. It is also conceivable that the protein conformation may be dependent on different binding partners in its native environment. Thus, PCA can be a very useful technique to help in this endeavor as it allows studying protein interactions within the cell.

It is plausible that the contribution of unstructured domains in Vif C-terminus for interaction with Gag/Gag–Pol and multimerization of Vif can be assessed in an environment where the conditional conformation of Vif is promoted (Henriet et al. 2009). Interaction of Vif with A3G, Gag, Gag–Pol, or HIV-1 genomic RNA has been shown to be mediated by protein domains scattered throughout its sequence. The hypothesis that the flexibility of these domains is important for binding different protein partners could be assessed by PCA in conditions where Gag/Gag–Pol proteins were present together with A3G and/or HIV-1 genomic RNA. This would allow a better understanding of Vif interaction with viral structural proteins. Therefore, β-lactamase fragment complementation presents itself as a suitable method to uncover the role of Vif domains in the cytoplasm or within virions.

Moreover, protein complementation can also be used for studying A3G interaction with Gag or cellular proteins. As showed in Results section, A3G–A3G interaction can be easily assessed by this method, and by using mutational analysis, it would be possible to study whether their interaction in the cytoplasm is similar to that into virions.

One can also imagine the possibility of using this technology to identify different cellular partners of Vif and A3G by screening and selecting cDNA libraries that complement an active β-lactamase (Pelletier et al. 1999). Nevertheless, some issues should be addressed with this system concerning the orientation of protein–protein interaction. This is of major importance since the method of β-lactamase fragment complementation presented here only allows parallel orientation of protein domains (Michnick et al. 2000). This weakness may be translated in reduced sensitivity of the method to detect specific protein–protein interactions.

In addition, major concern should be addressed when co-expressing Vif and A3G, since it was elegantly shown by Mercenne and co-workers that HIV-1 Vif binds to A3G 5′ UTR mRNA and inhibits deaminase translation (Mercenne et al. 2009). Thus, it is advisable to express A3G in these studies with an expression vector coding A3G gene sequence alone.

The data presented here show that the β-lactamase fragment complementation assay is effectively applicable to the intracellular assessment of Vif interaction with A3G and other members of the APOBEC family. Therefore, we propose that PCA could be used in the future in conjunction with other binding assays or as an alternative method for detection of Vif–A3 complexes in cellular lysates. This technique was also shown to be highly reproducible with a low probability of false-negatives. Furthermore, we believe that it could be used in the future to clarify on specific domains that may be involved in the interaction between Vif and APOBEC proteins and in drug screening systems.

Acknowledgments We would like to thank Dr. Stephen Michnick for providing the plasmids: pcDNA3.1-ZIP-15a.a-BLF1 (ZIP-B1) and pcDNA3.1-ZIP-15a.a-BLF2 (ZIP-B2). We would like to thank Dr. K. Strebel for providing the plasmid pcDNA3.1-A3G-HA.

Competing interests The authors declare that they have no competing interests.

References

Auclair JR, Green KM, Shandilya S, Evans JE, Somasundaran M, Schiffer CA (2007) Mass spectrometry analysis of HIV-1 Vif reveals an increase in ordered structure upon oligomerization in regions necessary for viral infectivity. Proteins 69:270–284

Balaji S, Kalpana R, Shapshak P (2006) Paradigm development: comparative and predictive 3D modeling of HIV-1 virion infectivity factor (Vif). Bioinformation 1:290–309

Barraud P, Paillart JC, Marquet R, Tisne C (2008) Advances in the structural understanding of Vif proteins. Curr HIV Res 6:91–99

Bebrone C, Moali C, Mahy F, Rival S, Docquier JD, Rossolini GM, Fastrez J, Pratt RF, Frère JM, Galleni M (2001) CENTA as a chromogenic substrate for studying beta-lactamases. Antimicrob Agents Chemother 45:1868–71

Bennett RP, Salter JD, Liu X, Wedekind JE, Smith HC (2008) APOBEC3G subunits self-associate via the C-terminal deaminase domain. J Biol Chem 283(48):33329–33336

Bergeron JR, Huthoff H, Veselkov DA, Beavil RL, Simpson PJ, Matthews SJ, Malim MH, Sanderson MR (2010) The SOCS-box of HIV-1 Vif interacts with ElonginBC by induced-folding to recruit its Cul5-containing ubiquitin ligase complex. PLoS Pathog 6:e1000925

Bernacchi S, Mercenne G, Tournaire C, Marquet R, Paillart JC (2010) Importance of the proline-rich multimerization domain on the oligomerization and nucleic acid binding properties of HIV-1 Vif. Nucleic Acids Res Nov 13 (In press)

Bertolaet BL, Knowles JR (1995) Complementation of fragments of triosephosphate isomerase defined by exon boundaries. Biochemistry 34:5736–5743

Bishop KN, Holmes RK, Sheehy AM, Davidson NO, Cho SJ, Malim MH (2004) Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr Biol 14:1392–1396

Bogerd HP, Doehle BP, Wiegand HL, Cullen BR (2004) A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. Proc Natl Acad Sci USA 101:3770–3774

Conticello SG, Harris RS, Neuberger MS (2003) The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr Biol 13:2009–2013

de Prat GG, Ruiz-Sanz J, Fersht AR (1994) Generation of a family of protein fragments for structure-folding studies. 2. Kinetics of association of the two chymotrypsin inhibitor-2 fragments. Biochemistry 33:7964–7970

de Virgilio M, Kiosses WB, Shattil SJ (2004) Proximal, selective, and dynamic interactions between integrin alphaIIbbeta3 and protein tyrosine kinases in living cells. J Cell Biol 165:305–311

Donahue JP, Vetter ML, Mukhtar NA, D'Aquila RT (2008) The HIV-1 Vif PPLP motif is necessary for human APOBEC3G binding and degradation. Virology 377:49–53

Douaisi M, Dussart S, Courcoul M, Bessou G, Lerner EC, Decroly E, Vigne R (2005) The tyrosine kinases Fyn and Hck favor the recruitment of tyrosine-phosphorylated APOBEC3G into Vif-defective HIV- 1 particles. Biochem Biophys Res Commun 329:917–924

Galarneau A, Primeau M, Trudeau LE, Michnick SW (2002) Beta lactamase protein fragment complementation assays as in vivo and in vitro sensors of protein protein interactions. Nat Biotechnol 20:619–622

Ghosh I, Hamilton AD, Regan L (2000) Detecting protein-protein interactions with a green fluorescent protein fragment reassembly trap: scope and mechanism. J Am Chem Soc 122:5658–5659

Goncalves J, Jallepalli P, Gabuzda DH (1994) Subcellular localization of the Vif protein of human immunodeficiency virus type 1. J Virol 68:704–712

Henriet S, Mercenne G, Bernacchi S, Paillart JC, Marquet R (2009) Tumultuous relationship between the human immunodeficiency virus type 1 viral infectivity factor (Vif) and the human APOBEC-3G and APOBEC-3F restriction factors. Microbiol Mol Biol Rev 73:211–232

Holmes RK, Koning FA, Bishop KN, Malim MH (2007) APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. J Biol Chem 282:2587–2595

Hu CD, Chinenov Y, Kerppola TK (2002) Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. Mol Cell 9:789–798

Huthoff H, Malim MH (2007) Identification of amino acid residues in APOBEC3G required for regulation by HIV-1 Vif and virion encapsidation. J Virol 81(8):3807–3815

Indrani P, Cui J, Maynard EL (2006) Zinc binding to the HCCH motif of HIV-1 virion infectivity factor induces a conformational change that mediates protein-protein interactions. Proc Natl Acad Sci USA 103:18475–18480

Kao S, Khan MA, Miyagi E, Plishka R, Buckler-White A, Strebel K (2003) The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. J Virol 77(21):11398–11407

Kao S, Goila-Gaur R, Miyagi E, Khan MA, Opi S, Takeuchi H, Strebel K (2007) Production of infectious virus and degradation of APOBEC3G are separable functional properties of human immunodeficiency virus type 1 Vif. Virology 369(2):329–339

Kataropoulou A, Bovolenta C, Belfiore A, Trabatti S, Garbelli A, Porcellini S, Lupo R, Maga G (2009) Mutational analysis of the HIV-1 auxiliary protein Vif identifies independent domains important for the physical and functional interaction with HIV-1 reverse transcriptase. Nucleic Acids Res 37:3660–3669

Kobayashi M, Takaori-Kondo A, Miyauchi Y, Iwai K, Uchiyama T (2005) Ubiquitination of APOBEC3G by an HIV-1 Vif-Cullin5-Elongin B-Elongin C complex is essential for Vif function. J Biol Chem 280:18573–18578

Ladurner AG, Itzhaki LS, Gay GD, Fersht AR (1997) Complementation of peptide fragments of the single domain protein chymotrypsin inhibitor 2. J Mol Biol 273:317–329

Langlois MA, Beale RC, Conticello SG, Neuberger MS (2005) Mutational comparison of the single-domained APOBEC3C and double-domained APOBEC3F/G anti-retroviral cytidine deaminases provides insight into their DNA target site specificities. Nucleic Acids Res 33:1913–1923

Li J, Potash MJ, Volsky DJ (2004) Functional domains of APOBEC3G required for antiviral activity. J Cell Biochem 92:560–572

Liu B, Yu X, Luo K, Yu Y, Yu XF (2004) Influence of primate lentiviral Vif and proteasome inhibitors on human immunodeficiency virus type 1 virion packaging of APOBEC3G. J Virol 78:2072–2081

Liu B, Sarkis PT, Luo K, Yu Y, Yu XF (2005) Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5- ElonB/C E3 ubiquitin ligase. J Virol 79:9579–9587

Luker KE, Smith MC, Luker GD, Gammon ST, Piwnica-Worms H, Piwnica-Worms D (2004) Kinetics of regulated protein-protein interactions revealed with firefly luciferase complementation imaging in cells and living animals. Proc Natl Acad Sci USA 101:12288–12293

Luo K, Xiao Z, Ehrlich E, Yu Y, Liu B, Zheng S, Yu XF (2005) Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. Proc Natl Acad Sci USA 102:11444–11449

Lv W, Liu Z, Jin H, Yu X, Zhang L (2007) Three-dimensional structure of HIV-1 VIF constructed by comparative modeling and the function characterization analyzed by molecular dynamics simulation. Org Biomol Chem 5:617–626

Ma XY, Sova P, Chao W, Volsky DJ (1994) Cysteine residues in the Vif protein of human immunodeficiency virus type 1 are essential for viral infectivity. J Virol 68:1714–1720

Mangeat B, Turelli P, Liao S, Trono D (2004) A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J Biol Chem 279:14481–14483

Marin M, Rose KM, Kozak SL, Kabat D (2003) HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 9:1398–1403

Marin M, Golem S, Rose KM, Kozak SL, Kabat D (2008) Human immunodeficiency virus type 1 Vif functionally interacts with diverse APOBEC3 cytidine deaminases and moves them between cytoplasmic sites of mRNA metabolism. J Virol 82 (2):987–998

Matagne A, Lamotte-Brasseur J, Frere JM (1998) Catalytic properties of class A B-Lactamases: efficiency and diversity. Biochem J 330:581–598

Mehle A, Goncalves J, Santa-Marta M, McPike M, Gabuzda D (2004a) Phosphorilation of a novel SOCS box regulates assembly of the HIV-1 Vif Cul5 complex that promotes APOBEC3G degradation. Genes Dev 18:2861–2866

Mehle A, Strack B, Ancuta P, Zhang C, McPike M, Gabuzda D (2004b) Vif overcomes de innate antivirfal activity of APOBEC3G by promoting its degradation in the ubiquitin-proteassome pathway. J Biol Chem 279:7792–7798

Mehle A, Thomas ER, Rajendran KS, Gabuzda D (2006) A zinc binding region in Vif binds Cul5 and determines cullin selection. J Biol Chem 281:17259–17265

Mercenne G, Bernacchi S, Richer D, Bec G, Henriet S, Paillart JC, Marquet R (2009) HIV-1 Vif binds to APOBEC3G mRNA and inhibits its translation. Nucleic Acids Res 38:633–646

Michnick SW, Remy I, Campbell-Valois FX, Vallée-Bélisle A, Pelletier JN (2000) Detection of protein-protein interactions by protein fragment complementation strategies. Methods Enzymol 328:208–230

Miller JH, Presnyak V, Smith HC (2007) The dimerization domain of HIV-1 viral infectivity factor Vif is required to block virion incorporation of APOBEC3G. Retrovirology 4:81

Navarro F, Bolmman B, Chen H, Konig R, Yu Q, Chiles K, Landau NR (2005) Complementary function of the two catalytic domains of APOBEC3G. Virology 33:374–386

Opi S, Kao S, Goila-Gaur R, Khan MA, Miyagi E, Takeuchi H, Strebel K (2007) Human immunodeficiency virus type 1 Vif inhibits packaging and antiviral activity of a degradation-resistant APOBEC3G variant. J Virol 81:8236–8246

Paulmurugan R, Umezawa Y, Gambhir SS (2002) Noninvasive imaging of protein-protein interactions in living subjects by using reporter protein complementation and reconstitution strategies. Proc Natl Acad Sci USA 99:15608–15613

Pelletier JN, Campbell-Valois FX, Michnick SW (1998) Oligomerization domain-directed reassembly of active dihydrofolate reductase from rationally designed fragments. Proc Natl Acad Sci USA 95:12141–12146

Pelletier JN, Arndt KM, Pluckthun A, Michnick SW (1999) An in vivo library-versus-library selection of optimized protein-protein interactions. Nat Biotechnol 17:683–690

Philippon A, Dusart J, Joris B, Frère JM (1998) The diversity, structure and regulation of beta-lactamases. Cell Mol Life Sci 54 (4):341–346

Remy I, Michnick SW (1999) Clonal selection and in vivo quantification of protein interactions with protein-fragment complementation assays. Proc Natl Acad Sci USA 96:5394–5299

Remy I, Michnick SW (2001) Visualization of biochemical networks in living cells. Proc Natl Acad Sci USA 98:7678–7683

Remy I, Michnick SW (2004) Regulation of apoptosis by the Ft1 protein, a new modulator of protein kinase. Mol Cell Biol 24:1493–1504

Remy I, Wilson IA, Michnick SW (1999) Erythropoietin receptor activation by a ligand-induced conformation change. Science 283:990–993

Remy I, Montmarquette A, Michnick SW (2004) PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. Nat Cell Biol 6:358–365

Russell R, Pathak VK (2007) Identification of two distinct human immunodeficiency virus type 1 Vif Determinants for interactions with human APOBEC3G and APOBEC3F. J Virol 81(15):8201–8210

Santa-Marta M, Silva FA, Fonseca AM, Goncalves J (2005) HIV-1 Vif can directly inhibit apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G-mediated cytidine deamination by using a single amino acid interaction and without protein degradation. J Biol Chem 280:8765–8775

Schrofelbauer B, Chen D, Landau NR (2004) A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). Proc Natl Acad Sci USA 101:3927–3932

Schrofelbauer B, Senger T, Manning G, Landau NR (2006) Mutational alteration of human immunodeficiency virus type 1 Vif allows for functional interaction with nonhuman primate APOBEC3G. J Virol 80:5984–5991

Sheehy AM, Gaddis NC, Malim MH (2003) The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med 9:1404–1407

Shiba K, Schimmel P (1992) Functional assembly of a randomly cleaved protein. Proc Natl Acad Sci USA 89:1880–1884

Simon JH, Fouchier RA, Southerling TE, Guerra CB, Grant CK, Malim MH (1997) The Vif and Gag proteins of human immunodeficiency virus type 1 colocalize in infected human T cells. J Virol 71:5259–5267

Simon V, Zennou V, Murray D, Huang Y, Ho DD, Bieniasz PD (2005) Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog 1:e6

Spotts JM, Dolmetsch RE, Greenberg ME (2002) Time-lapse imaging of a dynamic phosphorylation-dependent protein-protein interaction in mammalian cells. Proc Natl Acad Sci USA 99:15142–15147

Stopak de Noronha C, Yonemoto W, Greene WC (2003) HIV-1 blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol Cell 12:591–601

Subramaniam R, Desveaux D, Spickler C, Michnick SW, Brisson N (2001) Direct visualization of protein interactions in plant cells. Nat Biotechnol 19:769–772

Taniuchi H, Anfinsen CB (1971) Simultaneous formation of two alternative enzymology active structures by complementation of two overlapping fragments of staphylococcal nuclease. J Biol Chem 246:2291–2301

Tasayco ML, Carey J (1992) Ordered self-assembly of polypeptide fragments to form nativelike dimeric trp repressor. Science 255:594–597

Tian C, Yu X, Zhang W, Wang T, Xu R, Yu XF (2006) Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. J Virol 80:3112–3115

Ullmann A, Jacob F, Monod J (1967) Characterization by in vitro complementation of a peptide corresponding to an operator-proximal segment of the beta-galactosidase structural gene of Escherichia coli. J Mol Biol 24:339–343

Wolfe LS, Stanley BJ, Liu C, Eliason WK, Xiong Y (2010) Dissection of the HIV Vif interaction with human E3 ubiquitin ligase. J Virol 84:7135–7139

Xiao Z, Ehrlich E, Luo K, Xiong Y, Yu XF (2007) Zinc chelation inhibits HIV Vif activity and liberates antiviral function of the cytidine deaminase APOBEC3G. FASEB J 21:217–222

Xu H, Svarovskaia ES, Barr R, Zhang Y, Khan MA, Strebel K, Pathak VK (2004) A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor induced depletion. Proc Natl Acad Sci USA 101:5652–5657

Yang S, Sun Y, Zhang H (2001) The multimerization of human immunodeficiency virus type I Vif protein: a requirement for Vif function in the viral life cycle. J Biol Chem 276:4889–4893

Yang B, Gao L, Li L, Lu Z, Fan X, Patel CA, Pomerantz RJ, DuBois GC, Zhang H (2003) Potent suppression of viral infectivity by the peptides that inhibit multimerization of human immunodeficiency virus type 1 (HIV-1) Vif proteins. J Biol Chem 278:6596–6602

Yu X, Yu Y, Liu B, Luo K, Kong W, Mao P, Yu XF (2003) Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science 302:1056–1060

Yu Q, Chen D, Konig R, Mariani R, Unutmaz D, Landau NR (2004a) APOBEC3B and APOBEC3C are potent inhibitors of simian immunodeficiency virus replication. J Biol Chem 279:53379–53386

Yu Y, Xiao Z, Ehrlich ES, Yu X, Yu XF (2004b) Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev 18:2867–2872

Zennou V, Bieniasz PD (2006) Comparative analysis of the antiretroviral activity of APOBEC3G and APOBEC3F from primates. Virology 349:31–40

Zhang L, Saadatmand J, Li X, Guo F, Niu M, Jiang J, Kleiman L, Cen S (2008) Function analysis of sequences in human APOBEC3G involved in Vif-mediated degradation. Virology 370:113–121