Virology 387 (2009) 127–135

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Fragile X mental retardation protein restricts replication of human immunodeficiency virus type 1

Qinghua Pan [a], Liwei Rong [a], Xiaoyan Zhao [a], Chen Liang [a,b,*]

[a] McGill AIDS Centre, Lady Davis Institute-Jewish General Hospital, 3755 Cote Ste-Catherine Road, Montreal, Quebec, Canada H3T 1E2
[b] Department of Medicine, McGill University, Montreal, Quebec, Canada H3A 2B4

ARTICLE INFO

Article history:
Received 28 October 2008
Returned to author for revision
21 November 2008
Accepted 3 February 2009
Available online 27 February 2009

Keywords:
HIV-1
FMRP
Virus infectivity

ABSTRACT

Gag protein is the major structural component of human immunodeficiency virus type 1 (HIV-1) particles and drives virus assembly on cellular membranes. This function of Gag is attributed to its intrinsic self-multimerization ability as well as its interaction with cellular factors such as TSG101 that binds to the PTAP sequence in the p6 region of Gag and promotes HIV-1 budding through recruiting the ESCRT (endosomal sorting complex required for transport). As a result of its essential role in virus assembly, Gag also becomes the target of cellular restriction factors such as APOBEC3G and Trim5α. In this study, we report that the fragile X mental retardation protein (FMRP) interacts with HIV-1 Gag and is packaged into virus particles. Although knockdown of FMRP does not markedly affect production of virus particles, infectivity of HIV-1 virions was significantly decreased. Consistent with this observation, overexpression of the wild type FMRP, but not the FMRP mutants that lack the KH1 or the KH2 domains, led to 2- to 3-fold reduction of virus infectivity. Together, these results suggest that FMRP diminishes HIV-1 infectivity through association with viral Gag protein and virus particles.

Introduction

Human immunodeficiency virus type 1 (HIV-1) belongs to the retrovirade family. After entering the cell, HIV-1 RNA is converted to double-stranded viral cDNA within the cytoplasm by viral reverse transcriptase. This viral cDNA is then translocated to the nucleus where it is integrated into cellular chromosomal DNA by viral integrase and becomes a provirus. HIV-1 RNA is synthesized by cellular RNA polymerase II using proviral DNA as the template, and undergoes maturation such as acquiring the 5′ cap and the 3′ poly(A) tail and splicing. Only when viral Rev protein is made from multiply spliced viral RNA can the unspliced and singly spliced HIV-1 RNA molecules leave the nucleus and direct production of viral structural proteins (Frankel and Young, 1998).

HIV-1 RNA becomes coated with proteins during transcription, a process that occurs to all RNA species in cells. Composition of HIV-1 RNA–protein (RNP) complex changes frequently as viral RNA undergoes a series of metabolic processes (Cochrane et al., 2006). This reflects a requirement for a distinct set of proteins to assist viral RNA to accomplish each of the different tasks, such as splicing, nuclear export, transport to subcellular locations, translation and incorporation into virus particles. HIV-1 Gag protein contains the nucleocapsid (NC) sequence that specifically recognizes the cis-acting RNA signals present in HIV-1 full-length RNA, and thus recruits two copies into each virus particle (Berkowitz et al., 1996). Therefore, after its translation from the full-length HIV-1 RNA at the late phase of viral gene expression, Gag becomes the major component of viral RNP complex and as a result, considerably shapes the composition and functionality of viral RNP. Gag binding to viral RNA also marks the formation of viral RNP that is destined for packaging into virus particles (Freed, 1998). Characterizing the composition of this Gag–viral RNP complex has provided valuable information for understanding the activity of this complex such as assembling virus particles.

A number of cellular factors have been reported to interact with Gag and play various roles in HIV-1 assembly (Cantin et al., 2005). For example, TSG101 and ALIX/AIP1 proteins interact with the p6 domain of Gag and promote budding and release of virus particles from cell surface (Garrus et al., 2001; Strack et al., 2003). The AP-1 and AP-3 proteins bind to the matrix (MA) domain of Gag and also assist virus budding (Camus et al., 2007; Dong et al., 2005). Cyclophilin A interacts with the capsid (CA) domain of Gag and is involved in maturation of virus capsid structure (Gamble et al., 1996; Luban et al., 1993). As opposed to these aforementioned cellular factors that promote HIV-1 assembly, certain cellular proteins gain access to virus assembly complex by interacting with Gag and blocks HIV-1 replication. One example is APOBEC3G that interacts with the NC domain of Gag and thus gets packaged into progeny viruses in the absence of viral Vif protein. Once becoming part of viral reverse transcription complex, APOBEC3G abrogates generation of functional HIV-1 cDNA (Chiu and

* Corresponding author. McGill AIDS Centre, Lady Davis Institute-Jewish General Hospital, 3755 Cote Ste-Catherine Road, Montreal, Quebec, Canada H3T 1E2. Fax: +1 514 340 7537.
E-mail address: chen.liang@mcgill.ca (C. Liang).

0042-6822/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2009.02.006

Greene, 2008; Sheehy et al., 2002; Yu et al., 2003). Trim5α from the old world monkeys represents another example of this kind that targets the incoming HIV-1 core complex by recognizing the capsid protein and destroying viral reverse transcription complex (Sebastian and Luban, 2007; Stremlau et al., 2004).

Of Gag-associated cellular proteins is an RNA-binding protein named Staufen 1 that has been reported to interact with the NC domain of Gag and is also found in purified HIV-1 particles (Mouland et al., 2000). Staufen was first described in *Drosophila* to locate the *oskar* and *bicoid* mRNA to the posterior and anterior poles in the oocyte (St Johnston et al., 1991). Subsequently, two forms of Staufen were discovered in mammalian cells named Staufen 1 and Staufen 2 that mark the trafficking RNP granules in neurons (Miki et al., 2005). By associating with Staufen 1, it is postulated that HIV-1 Gag usurps the Staufen 1-containing cellular machinery to facilitate various activities of viral RNA such as transport, local translation, subcellular localization and packaging (Chatel-Chaix et al., 2004, 2007). Similar to Staufen, fragile X mental retardation protein (FMRP) also associates with the trafficking RNP granules in neurons and is actively involved in RNA transport and local translation (Bagni and Greenough, 2005; Darnell et al., 2005b). For example, FMRP has been reported to act as a translation suppressor and participates in inhibiting translation during RNA transport (Garber et al., 2006; Khandjian et al., 1996, 2004; Zalfa et al., 2003, 2006). The direct involvement of FMRP in RNA trafficking is supported by a recent finding that FMRP interacts with the KIF3C kinesin and thus acts as the adaptor between RNP granule and microtubules (Davidovic et al., 2007). Interestingly, FMRP and Staufen 1 are partially co-localized in cells and also represent the common components of specific RNP granules (Barbee et al., 2006). Consistent with this notion, FMRP- and Staufen-marked RNP complexes share at least 15% of their mRNA targets (Furic et al., 2007). In light of the important roles of both Staufen and FMRP in regulating post-transcriptional activity of RNA and their intimate relationship, we have investigated the possible interaction of FMRP with HIV-1. Our results demonstrated that, as opposed to the positive role of Staufen in HIV-1 replication, FMRP evidently decreases the infectivity of HIV-1 virions.

Results

FMRP is co-immunopurified with HIV-1 Gag protein

To immunopurify Gag protein from transfected 293T cells, we chose to use the Gag-TAP DNA construct that has the TAP sequence attached to the 3′ terminus of Gag (Fig. 1A) (Roy et al., 2006). TAP consists of two tag sequences including the protein A motif and the calmodulin-binding domain that are separated by the TEV protease cleavage site (Roy et al., 2006). This configuration allows purification of Gag protein by two consecutive affinity steps using IgG sepharose and calmodulin sepharose. Assessing the purified Gag complexes by Western blotting using anti-FMRP antibody revealed that endogenous FMRP was co-purified with Gag (Fig. 1A), indicating an association between FMRP and HIV-1 Gag. We next tested a panel of Gag mutants that are deleted of amino acids from the C-terminus (Fig. 1A). The results showed that deletion of the p6 sequence did not affect the level of FMRP co-precipitated with Gag; however, a further removal of the second zinc finger motif of NC domain drastically diminished Gag-FMRP association (Fig. 1A). To verify this critical role of NC in the interaction of Gag with FMRP, we replaced the NC sequence with leucine zipper motif from yeast transcription factor GCN4 in the context of BH10-Gag-TAP DNA and generated the BH10-Gag(LZ)-TAP DNA construct (Fig. 1B) (Roy et al., 2006). The wild type Gag-TAP and the Gag(LZ)-TAP mutant were purified as described above and assessed by Western blotting using anti-HIV-1 p24 and anti-FMRP antibodies. The results showed that FMRP was co-precipitated with the wild type Gag but not with the Gag(LZ) mutant (Fig. 1B).

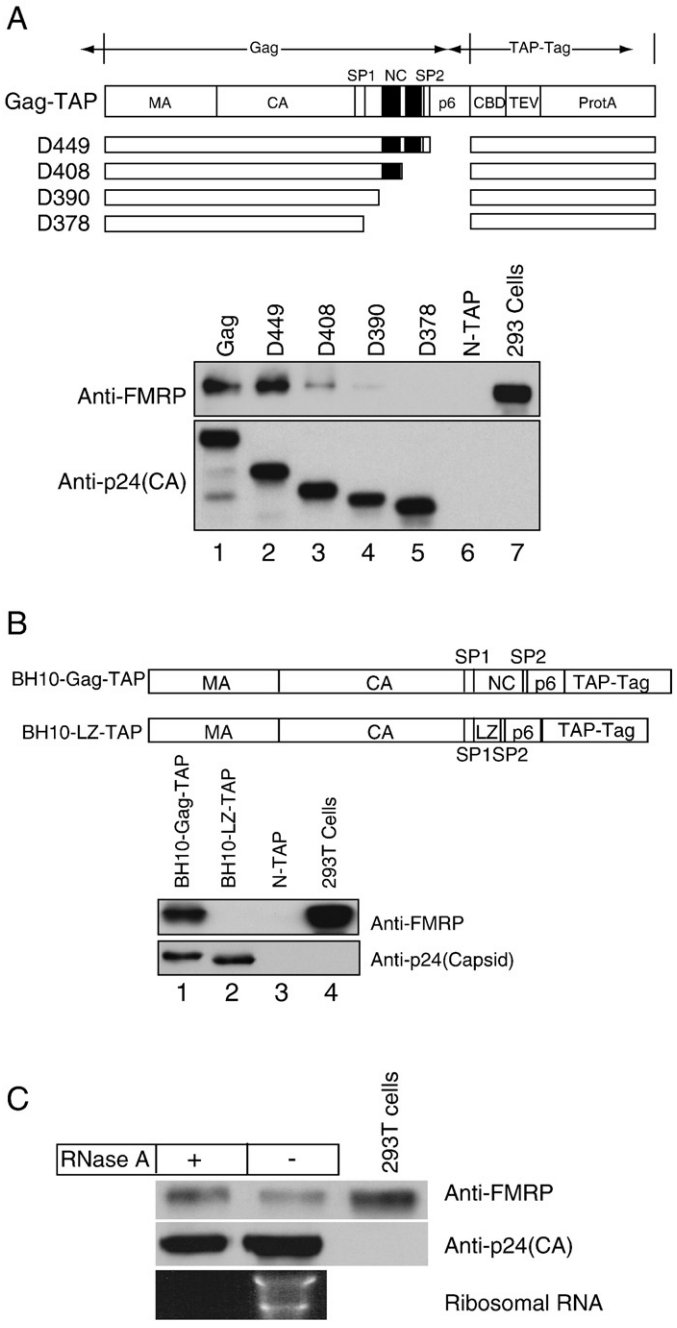

Fig. 1. FMRP associates with HIV-1 Gag protein. (A) Co-immunoprecipitation of FMRP with Gag protein. Gag-TAP protein has the TAP sequence attached at the C-terminus of Gag. TAP consists of the calmodulin binding domain (CBD), the TEV protease cleavage site and the protein A motif. The wild type and mutated Gag-TAP DNA constructs were transfected into 293T cells. Gag and its mutants were immuno-purified using the IgG sepharose and the calmodulin sepharose, followed by Western blotting using anti-HIV-1 p24(CA) and anti-FMRP antibodies. The N-TAP DNA expresses the TAP peptide and was employed as a control. (B) The Gag(LZ) mutant does not interact with FMRP. The TAP sequence is inserted at the C-terminus of Gag protein in the context of HIV-1 proviral DNA clone BH10-PR^{-} (protease-negative) to generate BH10-Gag-TAP. The NC sequence is replaced with the leucine zipper motif from yeast GCN4 transcription factor to create the BH10-LZ-TAP DNA. 293T cells were transfected with the BH10-Gag-TAP, BH10-LZ-TAP or N-TAP DNA. After affinity purification with IgG and calmodulin sepharoses, the materials were subjected to Western blotting using anti-HIV-1 p24(CA) or anti-FMRP antibodies. (C) RNase A treatment does not disrupt FMRP and Gag interaction. The amount of ribosomal RNA was monitored by Ethidium Bromide staining following electrophoresis in 1% agrose gels.