Virology 435 (2013) 329–340

Contents lists available at SciVerse ScienceDirect

Virology

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

# HIV-1 pre-mRNA commitment to Rev mediated export through PSF and Matrin 3

Anna Kula, Lavina Gharu, Alessandro Marcello*

*The Laboratory of Molecular Virology, International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34012 Trieste, Italy*

ARTICLE INFO

*Article history:*
Received 22 June 2012
Returned to author for revisions
16 August 2012
Accepted 21 October 2012
Available online 13 November 2012

*Keywords:*
Gene expression
HIV-1
Nucleus
RNA binding protein
RNA processing
Matrin 3
PSF
Rev

ABSTRACT

Human immunodeficiency virus gene expression and replication are regulated at several levels. Incompletely spliced viral RNAs and full-length genomic RNA contain the RRE element and are bound by the viral *trans*-acting protein Rev to be transported out of the nucleus. Previously we found that the nuclear matrix protein MATR3 was a cofactor of Rev-mediated RNA export. Here we show that the pleiotropic protein PSF binds viral RNA and is associated with MATR3. PSF is involved in the maintenance of a pool of RNA available for Rev activity. However, while Rev and PSF bind the viral pre-mRNA at the site of viral transcription, MATR3 interacts at a subsequent step. We propose that PSF and MATR3 define a novel pathway for RRE-containing HIV-1 RNAs that is hijacked by the viral Rev protein.
© 2012 Elsevier Inc. All rights reserved.

## Introduction

Human immunodeficiency virus type 1 (HIV-1) gene expression and replication are regulated at the transcriptional and post-transcriptional level. The generation of infectious viral progeny requires the synthesis and export to the cytoplasm of several fully spliced subgenomic mRNAs encoding for regulatory factors that include the Tat transactivator and Rev (Greene and Peterlin, 2002). Rev promotes the expression of Gag, Pol and Env incompletely spliced viral RNAs and full-length genomic RNAs (Malim et al., 1989; Sodroski et al., 1986) reviewed in: Kula and Marcello (2012), Pollard and Malim (1998), Yedavalli and Jeang (2011b). Rev promotes the export of this class of RNAs from the nucleus through the association with an RNA element called the Rev response element (RRE) that is present in the *env* gene (Chang and Sharp, 1989; Kjems et al., 1991; Zapp and Green, 1989). HIV-1 exploits a variety of mechanisms to make sure that all required RNAs are balanced for an efficient life cycle (Purcell and Martin, 1993; Schwartz et al., 1990). Sub-optimal splice acceptor sequences and the activity of splicing regulators reduce HIV-1 pre-mRNA splicing rates (Amendt et al., 1994, 1995; Caputi et al., 1999; Dyhr-Mikkelsen and Kjems, 1995; O'Reilly et al., 1995; Si et al., 1997; Staffa and Cochrane, 1994, 1995; Tange et al., 2001). This mechanism is believed to permit the production of both fully spliced mRNAs as well as RRE-containing RNAs. HIV-1 also encodes for regulatory sequences called the instability (INS) sequences (Cochrane et al., 1991; Maldarelli et al., 1991; Nasioulas et al., 1994; Schneider et al., 1997; Schwartz et al., 1992). One possible role for these elements is to ensure that incompletely spliced pre-mRNAs are not simply degraded in the nucleus prior to export to the cytoplasm by preserving the RNA in nuclear sub-compartments (Berthold and Maldarelli, 1996; Chang and Sharp, 1989; Mikaelian et al., 1996). Hence, a combination of *cis*-acting RNA sequences with a *trans*-acting viral protein (Rev) together with host factors concur to regulate HIV-1 gene expression and replication.

The molecular details of Rev binding to RRE as well as the mechanism of Rev-mediated RNA export by Exportin 1 (CRM1) have been described in detail (Daugherty et al., 2010; Fornerod et al., 1997; Kjems et al., 1991; Malim and Cullen, 1991). However, much less is known of the nuclear steps that occur between pre-mRNA biogenesis, Rev recognition and nuclear export. To address this issue we have recently initiated an unbiased program of discovery of nuclear factors that associated with HIV-1 RNA (Kula et al., 2011). Among them Matrin 3 (MATR3) was found to be specifically associated with the viral RNA, as confirmed also by other groups (Naji et al., 2011; Yedavalli and Jeang, 2011a). MATR3 had no effect on transcription from the HIV-1 LTR but promoted the Rev-dependent cytoplasmic accumulation and consequent translation of RRE-containing

* Corresponding author. Fax: +39 040226555.
*E-mail address:* marcello@icgeb.org (A. Marcello).

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.10.032

unspliced RNAs (Kula et al., 2011; Yedavalli and Jeang, 2011a). MATR3 is a RNA binding protein and a component of the inner nuclear matrix (Belgrader et al., 1991; Hibino et al., 2006; Nakayasu and Berezney, 1991; Zeitz et al., 2009). Together with the cellular protein polypyrimidine tract-binding protein associated binding factor PSF (also known as splicing factor Proline-Glutamine Rich, SFPQ), MATR3 has been implicated in the nuclear retention of hyperedited mRNA (Prasanth et al., 2005; Zhang and Carmichael, 2001). PSF is a multifunctional nuclear protein that has been involved in a variety of processes in addition to nuclear retention of hyperedited RNA including splicing and transcription (Emili et al., 2002; Kaneko et al., 2007; Shav-Tal and Zipori, 2002). PSF has also been shown to bind specifically to HIV-1 INS elements (Zolotukhin et al., 2003). PSF was also found associated with HIV-1 RNA in the nucleus but it's role in HIV-1 gene expression remained unknown (Kula et al., 2011).

In this work we investigated the role of PSF in Rev-mediated RNA export and its relationship with MATR3. We found that PSF is not involved in LTR-mediated transcription but is required for Rev activity independent of A to I hyperediting of HIV-1 RNA. PSF and MATR3 interacted at the protein level while their interaction with Rev occurred only through RNA. PSF and Rev associated with nascent pre-mRNA at the HIV-1 transcription site while MATR3 bound viral unspliced RNA in the insoluble nuclear matrix. Since PSF was shown to bind the INS elements (Zolotukhin et al., 2003) and MATR3 was shown to promote Rev-mediated export of unspliced RNA (Kula et al., 2011), we propose that HIV-1 unspliced RRE-containing HIV-1 RNAs are committed to MATR3/Rev mediated export by PSF.

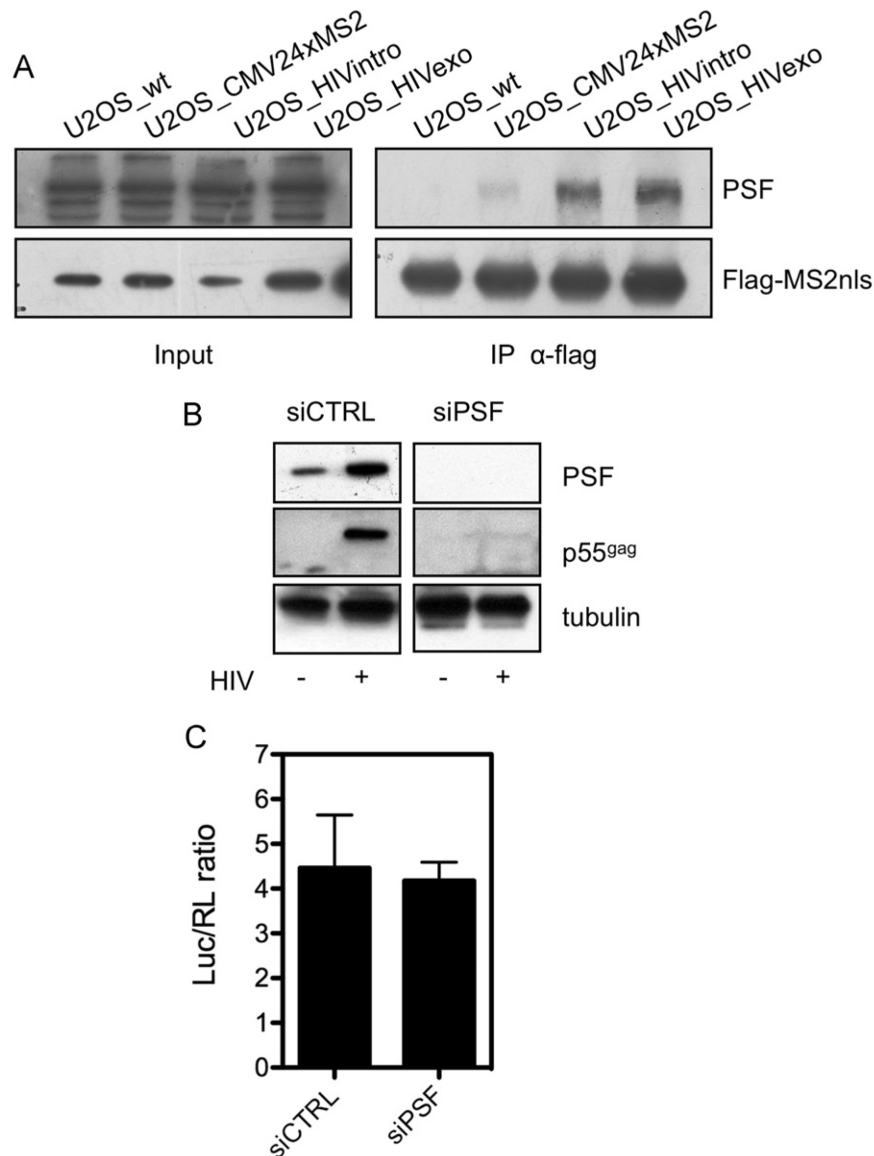


**Fig. 1.** PSF binds HIV-1 RNA and acts at the post-transcriptional level. (A) Pulldown of HIV-1 RNA and endogenous PSF. Whole cell extracts from mock cells (U2OS_wt), U2OS_HIVexo, U2OS_HIVintro or U2OS_CMV24xMS2 expressing Flag-MS2nls and Tat were immunoprecipitated with anti-flag antibody. Immunoblot analysis was then performed to detect the RNA-bound endogenous PSF (IP). Immunoblots for the whole cell extracts against PSF and flag-MS2nls (input) are also shown. (B) PSF knockdown leads to a decrease of Gag expression. HeLa cells were transfected with the siRNA targeting PSF (siPSF) or with a control siRNA (siCTRL). After 48 h siRNA-transfected cells were co-transfected with pNL4.3R-E-luc and harvested 24 h later for immunobloting. Tubulin is the protein loading control. (C) PSF knockdown does not affect the luciferase activity. HeLa cells were transfected with the indicated siRNAs. After 48 h siRNA-transfected cells were co-transfected with pCMV-Renilla together with the pNL4.3R-E-luc HIV molecular clone and harvested 24 h later for luciferase assays. Relative Luc/RL expression was normalized to protein levels measured by Bradford assay. The results of three independent experiments are shown ± SD.

Download English Version:

https://daneshyari.com/en/article/3424180

Download Persian Version:

https://daneshyari.com/article/3424180

Daneshyari.com