COMMENTARY

Cleavage, a real turn-off? HIV-mediated proteolysis of PABP1

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In this issue of the *Biochemical Journal*, Álvarez and colleagues have identified PABP1 [poly(A)-binding protein 1] as a target of protease cleavage during HIV infection. The study shows that HIV-1, HIV-2 and mouse mammary tumour virus, but not other retroviruses, target PABP1 for cleavage and identifies cleavage sites within the RNA-recognition motifs and C-terminal region of the protein. This suggests that PABP1 cleavage may be important in the shut-off of host translation during HIV infection. This extends the viral families that are known to target PABP1 to include *Retroviridae*, suggesting that PABP1 may be a central target of viral infection.

Key words: HIV infection, host translation shut-off, host–viral interaction, poly(A)-binding protein (PABP), proteolysis.

Translational control of mRNAs is central to the regulation of gene expression and is important in the aetiology of human disease. Many viruses have evolved complex mechanisms to inhibit host cellular mRNA translation while facilitating efficient translation of their genome, thereby ensuring maximum production of viral products [1]. The study of viral translation has revealed important information pertaining not only to viral life cycles, but also to many critical facets of cellular translational control.

Whereas most cellular mRNAs initiate translation via a cap-dependent pathway, many viral mRNAs utilize IRESs (internal ribosome entry sites) [1]. Cap-dependent translation starts with the recognition of the 5′ cap by a complex of proteins. This leads to the recruitment of the small ribosomal subunit, which migrates to the initiator codon where the large ribosomal subunit joins. Although IRES-dependent translation differs from this pathway to various degrees, all IRESs function in a cap-independent manner, recruiting ribosomal subunits at an internal site [2]. Differences in the factor requirements of these pathways allow viral translation when cap-dependent translation is inhibited.

In recent years, a number of studies have revealed a cytoplasmic PABP [poly(A)-binding protein], PABP1, as an important viral target [1,3]. PABP1 binds to poly(A) tails at the 3′ end of mRNAs and plays critical roles in mRNA translation and stability [3]. PABP1 contains four non-identical RRMs (RNA-recognition motifs), which have different specificities for RNA, and a C-terminal region that does not bind RNA. This C-terminal region is composed of a proline-rich region, which is important for PABP1–PABP1 interactions, and a helical domain (PABC domain). The RRMs and the PABC domain interact with translation initiation factors eIF4G (eukaryotic initiation factor 4G) (RRM1 and RRM2), eIF4B (PABC domain) and the termination factor eRF3 (eukaryotic release factor 3) (PABC domain) [3]. Interactions between PABP1 and initiation factors bound to the 5′ end of the mRNA are proposed to circularize mRNAs, thereby enhancing translation initiation. In particular, the interaction between PABP1 and eIF4G appears to be critical for translation [3]. The eIF4G–PABP1 interaction is thought to enhance the affinity of the cap-binding complex (eIF4A–eIF4G–eIF4E) for the 5′ cap and PABP1 for the poly(A) tail. This results in more efficient recruitment of the small ribosomal subunit. Interaction with eIF4B is also thought to enhance this step, whereas interaction with eRF3 may promote ribosome recycling or link translation to mRNA degradation [3].

Factors involved in cap-dependent initiation are often modified or hijacked during viral infection [1]. In rotavirus infection, binding of the viral protein NSP3 (non-structural protein 3) to eIF4G prevents PABP1 from interacting with proteins bound to the cap, leading to a decrease in the translation of adenylated cellular mRNAs [1]. NSP3 promotes viral translation by binding to unadenylated viral 3′ ends, putatively circularizing the mRNA via the formation of an alternative end-to-end complex [1].

PABP1 is also targeted during picornavirus infections (e.g. by poliovirus and Coxsackie B3 virus) [1] and by caliciviruses [4]. In contrast with rotavirus, these viruses express proteases that cleave translation factors, including PABP1. Poliovirus encodes two proteases that cleave either at a single site (2A protease) or multiple sites (3C protease) within the C-terminal region of PABP1 [1,3,5]. The use of compounds that block the cleavage of PABP1 suggest that it is important for complete host translation shut-off, which also requires eIF4G cleavage [5]. Caliciviruses encode a 3C-like protease that cleaves the C-terminal region of PABP1, but not eIF4G; however, the exact cleavage site varies between family members [4]. Importantly, in feline calicivirus-infected cells, the kinetics of PABP1 cleavage is concomitant with the decrease in host protein synthesis [4]. A direct role for PABP1 is further suggested by observations that cleavage of PABP1 by the calicivirus 3C-like protease, similar to poliovirus 3C protease, inhibits translation of polyadenylated, but not unadenylated, mRNAs *in vitro*. Critically, this translational inhibition is rescued by the addition of recombinant PABP1, suggesting that cleavage of PABP1 is important for this effect [4].

HIV belongs to the *Lentivirinae* subfamily of *Retroviridae*, and is the causative agent of AIDS. HIV-1 is found worldwide, whereas the antigenically distinct HIV-2 is prevalent in West Africa [6].

HIV gene expression involves a complex interplay between viral transcription, alternative splicing, nucleo-cytoplasmic transport and translation, which is often regulated by intrinsic viral regulatory sequences and viral proteins. Transcription enhanced by the viral protein Tat leads to the synthesis of 30 different

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viral mRNAs. These viral transcripts include genomic RNAs of ∼9 kb, singly spliced mRNAs of ∼4 kb and multiply spliced mRNAs of ∼2 kb. These are derived by alternative splicing of a single full-length transcript, generating mRNAs with common 5′ and 3′ ends, which are polyadenylated at the 3′ end [6]. Both singly spliced and unspliced viral mRNAs contain a RRE (Rev-responsive element) that facilitates the export of intron-containing mRNAs via the binding of the viral protein Rev. Transport of intron-containing mRNAs to the cytoplasm ensures that viral mRNAs can be translated and later packaged into mature virions [6].

As with other viruses, retroviruses also repress host cell translation in favour of enhancing the translation of viral mRNAs. Similar to picornaviruses, retroviruses, including HIV-1, HIV-2, MMTV (mouse mammary tumour virus), MoMLV (Moloney murine leukaemia virus), HTLV-1 (human T-cell leukaemia virus type 1) and SIV (simian immunodeficiency virus), cleave eIF4GI, inhibiting cap-dependent translation [7,8]. Under these conditions, HIV-1 translation may be facilitated by multiple IRESs [9]. However, the *trans*-acting factors required for HIV IRES-mediated translation have not been fully characterized.

A number of HIV proteins that enhance the translation of viral mRNAs have also been identified [10]. In particular, Rev has been shown to promote the loading of Gag/Pol and Vpu/Env mRNAs on to polysomes [11]. Other HIV proteins, including Tat, Gag precursor polypeptide, matrix and nucleocapsid proteins, also appear to play potential roles in enhancing the translation of viral mRNAs while repressing the translation of host cell protein synthesis [10].

In this issue of the *Biochemical Journal*, Álvarez and colleagues have reported that HIV proteases cleave PABP1 [8]. Although the overall reduction in full-length PABP1 is modest in infected cells, cleavage products were detected. Electroporation of viral protease RNA into cells showed that HIV protease, and not other viral proteins, was responsible for PABP1 cleavage. Proteolytic cleavage of PABP1 was observed only in the presence of HIV-1, HIV-2 or MMTV proteases. Interestingly, those retroviral proteases that showed no cleavage of PABP1, including MoMLV, HTLV-1 and SIV, all had a substantial effect on the cleavage of eIF4GII as well as eIF4GI. This suggests that the cleavage of translation initiation factors is evolutionarily conserved across the *Retroviridae* family.

The kinetic activity of HIV-1 and HIV-2 proteases on PABP1 is similar to their activity on eIF4GI in cell culture and in a cell-free system. However, HIV-2 protease cleaves both PABP1 and eIF4GI more efficiently than HIV-1 protease, suggesting some sequence variation affecting the catalytic activity of the enzyme. Mapping of the HIV-1 and HIV-2 protease cleavage sites found that both cleave PABP1 around the same sites. HIV-1 protease cleaves twice at residues 237 and 477, and HIV-2 protease cleaves three times at residues 237, 410 and 477. This cleaves PABP1 in the RRM3, a site not previously characterized as a target for viral protease, and in the C-terminal region, a region synonymous with cleavage by other viral proteases. The conservation of HIV cleavage sites in PABP1 supports the idea that it is a genuine substrate for the viral proteases.

The observation that cleavage of PABP1 and eIF4GI occur with similar kinetics [8] raises the interesting hypothesis that PABP1 cleavage could contribute to host translation shut-off during HIV infection. It is now important to directly determine if PABP1 cleavage contributes to a loss of host cell translation. As with caliciviruses and enteroviruses, cleavage of PABP1 in HIV-infected cells is not complete. This raises questions about the relative contribution of PABP1 cleavage to host translational shut-off by HIV. Nonetheless, cleavage of PABP1 may lead to a loss of translation by separating the C-terminal region, which binds eIF4B, eRF3 and other PABP1 molecules, from the RRM-containing region, which binds eIF4G and poly(A) tails. It is not clear whether the long and short N-terminal fragments retain some function via eIF4G, as loss of the C-terminal region may result in an insufficient affinity to achieve poly(A)-binding *in vivo*. It is possible that fragments of cleaved PABP1 may function as dominant-negative inhibitors of translation by actively sequestering translation factors such as eIF4G, eIF4B and eRF3 away from polyadenylated mRNAs. However, these fragments are not always detected and may not be stable, suggesting that they might not contribute directly to the shut-off of host translation.

Given that HIV mRNAs are polyadenylated, it might seem surprising that HIV would cleave PABP1, as this would be predicted to also target HIV translation. One possibility is that, while HIV mRNAs are polyadenylated, translation from HIV IRESs is not responsive to poly(A), or that HIV IRESs are stimulated via a mechanism that does not require PABP1–eIF4G interactions, as described for some cellular IRESs [12]. Alternatively, a subset of the fragments cleaved from PABP1 may be recruited by HIV to drive the translation of HIV mRNAs, in a manner similar to eIF4G cleavage products in IRES-mediated translation. Recruitment to HIV mRNAs may stabilize a portion of these PABP1 fragments from degradation, which accounts for their detection. This hypothesis is supported by observations that tethering of different fragments of PABP1 can drive translation, in some cases as efficiently as the full-length protein [13]. Thus, if HIV can recruit PABP1 fragments via a preferential RNA-binding site or via a protein–protein interaction that recruits a fragment to the mRNA indirectly, this could result in efficient enhancement of HIV translation. A similar mechanism could drive efficient competition of viral mRNAs for the reduced levels of full-length PABP1. Indeed, recruitment of PABPs to mRNAs by mRNA-specific binding proteins has recently been shown to promote the translation of some cellular mRNAs [14]. Interestingly, it has been previously proposed that the viral protein Rev promotes recruitment of PABP1 to Rev-dependent RNAs [15]. It is therefore conceivable that Rev could directly recruit full-length or fragments of PABP1 to viral mRNAs. However, a direct interaction between HIV proteins and PABP1 has not yet been demonstrated. Further insights into the role of HIV-mediated PABP1 cleavage in host translation shut-off may highlight PABP1 as a central target during viral infection. Moreover, it may aid in a greater understanding of HIV pathogenicity, and allow greater diversification in the pursuit of alternative methods of antiviral therapy.

REFERENCES

1 Bushell, M. and Sarnow, P. (2002) Hijacking the translation apparatus by RNA viruses. J. Cell Biol. **158**, 395–399

2 Jackson, R. J. (2005) Alternative mechanisms of initiating translation of mammalian mRNAs. Biochem. Soc. Trans. **33**, 1231–1241

3 Gorgoni, B. and Gray, N. K. (2004) The role of cytoplasmic poly(A)-binding proteins in regulating gene expression: a developmental perspective. Brief Funct. Genomics Proteomics **3**, 125–141

4 Kuyumcu-Martinez, M., Belliot, G., Sosnovtsev, S. V., Chang, K. O., Green, K. Y. and Lloyd, R. E. (2004) Calicivirus 3C-like proteinase inhibits cellular translation by cleavage of poly(A)-binding protein. J. Virol. **78**, 8172–8182

5 Joachims, M., Van Breugel, P. C. and Lloyd, R. E. (1999) Cleavage of poly(A)-binding protein by enterovirus proteases concurrent with inhibition of translation *in vitro*. J. Virol. **73**, 718–727

6 Wang, W. K., Chen, M. Y., Chuang, C. Y., Jeang, K. T. and Huang, L. M. (2000) Molecular biology of human immunodeficiency virus type 1. J. Microbiol. Immunol. Infect. **33**, 131–140

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7 Álvarez, E., Menendez-Arias, L. and Carrasco, L. (2003) The eukaryotic translation initiation factor 4GI is cleaved by different retroviral proteases. J. Virol. **77**, 12392–12400
8 Álvarez, E., Castello, A., Menendez-Arias, L. and Carrasco, L. (2006) HIV protease cleaves poly(A)-binding protein. Biochem. J. **396**, 219–226
9 Brasey, A., Lopez-Lastra, M., Ohlmann, T., Beerens, N., Berkhout, B., Darlix, J. L. and Sonenberg, N. (2003) The leader of human immunodeficiency virus type 1 genomic RNA harbors an internal ribosome entry segment that is active during the G2/M phase of the cell cycle. J. Virol. **77**, 3939–3949
10 Thompson, S. R. and Sarnow, P. (2000) Regulation of host cell translation by viruses and effects on cell function. Curr. Opin. Microbiol. **3**, 366–370
11 D'Agostino, D. M., Felber, B. K., Harrison, J. E. and Pavlakis, G. N. (1992) The Rev protein of human immunodeficiency virus type 1 promotes polysomal association and translation of gag/pol and vpu/env mRNAs. Mol. Cell. Biol. **12**, 1375–1386
12 Thoma, C., Bergamini, G., Galy, B., Hundsdoerfer, P. and Hentze, M. W. (2004) Enhancement of IRES-mediated translation of the c-myc and BiP mRNAs by the poly(A) tail is independent of intact eIF4G and PABP. Mol. Cell **15**, 925–935
13 Gray, N. K., Coller, J. M., Dickson, K. S. and Wickens, M. (2000) Multiple portions of poly(A)-binding protein stimulate translation *in vivo*. EMBO J. **19**, 4723–4733
14 Collier, B., Gorgoni, B., Loveridge, C., Cooke, H. J. and Gray, N. K. (2005) The DAZL family of proteins are PABP binding proteins that regulate translation in germ cells. EMBO J. **24**, 2656–2666
15 Campbell, L. H., Borg, K. T., Haines, J. K., Moon, R. T., Schoenberg, D. R. and Arrigo, S. J. (1994) Human immunodeficiency virus type 1 Rev is required *in vivo* for binding of poly(A)-binding protein to Rev-dependent RNAs. J. Virol. **68**, 5433–5438

Received 11 April 2006; accepted 12 April 2006
Published on the Internet 15 May 2006, doi:10.1042/BJ20060545

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