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A Possible Innate Cellular Defense System against Viral Infection: PKR-Independent Translational Inhibition in Infected Cells by HIV-1 Tat Protein

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Summary

Viruses are obligatory intracellular parasite and use cellular biosynthetic machinery for replication. To replicate and cause disease, viruses must overcome cellular and humoral immune responses, defeat innate cellular defense systems, usurp cellular factors, and reprogram the normal biology of the cell. Recently, studies of innate antiviral and immune responses, host genetics, cell biology, and neuropathogenesis have identified host factors that viruses must overcome in order to replicate. The field is now poised to combine these studies to define the molecular mechanisms that underlie important host/virus interactions. Of those, studies of cellular factors and genetic elements that are involved in the innate cellular defense systems has been focused on the interferon-induced, dsRNA-dependent PKR/RNase L activation. It has been known well that the induction of interferon eventually causes non-specific translation initiation through the inactivation of eIF2-α and the degradation of mRNAs. During the course of evolution, viruses devised various tactics to redirect translational machinery and to circumvent host defenses.

In this study, we found that HIV-1 Tat inhibits translation irrelative to mRNA species. The Tat protein of HIV-1 has been known to be critical for viral replication. It binds to TAR RNA at viral long terminal repeat (LTR) promoter region and activates transcription several hundred-fold. In addition to stimulation of HIV-1 gene expression, accumulating evidence suggests that Tat may exert its effects on various cellular functions as a growth factor, a T-cell activatior, a regulator of gene expression, and an inducer or protector of cellular apoptosis. These findings have supported the speculation that Tat has pleiotropic non-transcriptional functions in the cells. To understand better the biology of the HIV-1 Tat in AIDS-pathogenesis, we have investigated translational effect of HIV-1_{BRU} Tat protein *in vitro* translation systems. Here we report that HIV-1_{BRU} Tat inhibits translation irrelative to mRNA species. The translational inhibition by Tat is independent of PKR activation, 5' capping of mRNA, or ribosome entry site. The full-length Tat protein expressed from two exons showed more dramatic reduction of translation than the first exon Tat protein. Interestingly, HIV-1_{HXB3} Tat has no effect on the *in vitro* translation. Also, a single amino acid change in the HIV-1_{BRU} Tat (K41E) blocked the translational inhibitory effect. These findings

suggest that translation may also be an important level of control in the pathogenesis by HIV-1 infection.

Results

Translation inhibition by HIV-1 Tat variants

Effect of HIV-1 Tat on translation was examined by *in vitro* assay using rabbit reticulocyte lysate and Xef-1 (Xenopus elongation factor-1) mRNA produced by *in vitro* transcription reaction. Translation inhibition by Tat proteins is shown in figure 2. In this assay, 0.2 μg of Tat was added to 20 μl of translation mixture. The first exon Tat and the full-length Tat of HIV-1 Bru type decreased translation efficiency about 60% and 80%, respectively. Full-length Tat inhibited translation reaction efficiently at lower concentrations. Fifty ng of full-length Tat reduced translation about 70 % whereas the first exon Tat merely affected the reaction. Both the first exon and the full-length forms of mutant tat proteins in which the 41st amino acid lysine was replaced with glutamate exerted no effect on translation efficiency. From these results it seems that the second exon of Tat protein is dispensable for inhibition of translation, although the presence of it augmented the inhibition potency. By the fact that the mutant was unable to inhibit translation, it can be inferred that the region containing the 41^{st} amino acid could interact with a cellular factor involved in regulation of translation. As well as mutant Tat proteins, the wild type Tat of HXB3 strain also exerted no effect on translation. The amino acid sequence of HXB3 type tat is different from Bru type at 5 points; 39^{th}, 58^{th}, 59^{th}, 61^{st}, and 67^{th}.

To examine whether the inhibitory effect of Tat protein is specific for the RNA tested, *in vitro* translation assay was also carried out with Env (envelope glycoprotein of HIV-1), EnvΔ24 (envelope glycoprotein without TAR-like sequence), AT (antitrypsin) and Luc (luciferase) RNAs. Although the extent of inhibition was varied between the different RNA species, the first exon Tat inhibited all of the translation reactions tested.

Translation block by HIV-1 Tat does not require 5'-capping of mRNA

A priori for eukaryotic cells to commence translation is recognition of 5' cap structure by eIF-4F. The cap dependence can be alleviated by lowering salt concentration in *in vitro* translation system using rabbit reticulocyte lysate. The presence of cap structure exerted no effect on translation inhibition by Tat. This result indicates that translation block by Tat is not on the recognition of the cap structure. This was also bolstered by the fact that the translation process from IRES (Internal Ribosome Entry Sequence) was also inhibited by Tat. A bicistronic mRNA in which IL-16 is synthesized by scanning of initiation complex from 5' end and HSV-1 thymidine kinase is produce after IRES was used for *in vitro* translation assay. In this system, Tat inhibited translation of both IL-16 and thymidine kinase.

HIV-1 Tat inhibits translation at initiation stage

Most of translational control events occur at the initiation stage. To find out if the translational

control by Tat also occurs at the initiation stage, mRNA was incubated with reticulocyte lysate before amino acid mixture and Tat were added. The pre-incubation of mRNA with lysate would allow an initiation complex to be formed before Tat acts upon it. The pre-incubation abolished the Tat-mediated translational block. From this it can be inferred that Tat inhibits translation by interacting with a cellular factor that affects formation of functional initiation complex.

PKR exerts no effect on Tat-mediated translational block

PKR has been known to block translation by phosphorylating eIF2-α in response to double-stranded RNA. PKR also interacts with and phosphorylate Tat, although the consequences of Tat phosphorylation have not been elucidated. Non-discriminating translational block by both proteins and interaction between them was followed by a speculation if Tat-mediated translational block has any relevance to PKR activity. Rabbit reticulocyte lysate was pre-treated with poly(I) · poly(C) or 2-aminopurine. poly(I) · poly(C) increase PKR activity whereas 2-aminopurine suppress it. The treated lysate was used for *in vitro* transcription assay in the presence or absence of Tat. Despite the interaction between PKR and Tat, PKR activity exerted no effect on Tat-mediated translational block. Use of wheat germ extract, in which PKR activity lacks, showed similar results. This indicates that translation block by Tat works independently of PKR-mediated translational shut-off.

Discussion

Selective translation of viral mRNA could confer replicative advantage by mobilizing cellular resources for production of viral proteins. But in this study, we observed that Tat inhibit translation irrespective of the mRNA species. Translation of HIV-1 RNA was equally inhibited as cellular mRNA. The host responds to infection by various measures and one of which is to shut off translation all together. Tat-mediated translational block likewise could be a host response against viral attack as in the case with PKR response. But it cannot be excluded that a viral protein other that Tat can confer selectivity to viral mRNA.

In our study, we observed that HIV-1 Tat of Bru strain, but not of HXB3, blocked translation. Non-responsiveness against HXB3 type Tat could be restricted to *in vitro* translation assays, and it could take effect in its natural host. Alternatively, if translation block by Tat arose as a part of host defense strategy, then the non-responsiveness could be a viral countermeasure.

The global down-regulation of translation is a consequence of PKR activation. In addition, Tat has been known to be phosphorylated by PKR. Thus, it seemed to be plausible to propose that Tat-mediated block was related to PKR. But our study showed up to the contrary. Enhancing or depressing PRK activity did not affected Tat-mediated translational block. Presence or absence of cap structure also exerted no effect. Thus phosphorylation of eIF2 or recognition of cap structure by eIF4 family of proteins are not seems to be target on which Tat acts. But the block was still imposed on initiation step. Identification of translational factor(s) affected by Tat should be carried out to elucidate the mechanism of Tat-mediated translational inhibition.