

INHIBITION OF HIV-1 mRNA 3' END CLEAVAGE BY eIF3f

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Viruses often use host machinery in unusual ways to execute different steps during their replication. To identify host factors critical for virus replication, we screened cDNA expression libraries for genes or gene fragments that interfere with HIV-1 vector transduction. Our most potent clone encoded the N-terminal 91 residues of the eukaryotic initiation factor 3 subunit f (N91-eIF3f).

Overexpression of N91-eIF3f or full-length eIF3f severely restricted the replication of the virus, by specifically targeting the 3' end of the viral mRNA. Proviruses were formed normally but viral mRNA levels were reduced in the nucleus and cytoplasm. Restoration of viral gene expression was achieved upon addition of a heterologous polyadenylation signal downstream from the 3' LTR. Further, we show that cleavage, rather than polyadenylation, at the 3' end of HIV mRNAs is reduced in the presence of N91-eIF3f and eIF3f *in vitro*. Additionally, in the absence of endogenous eIF3f, the N91-eIF3f fragment is unable to restrict the virus.

A possible mechanism by which N91-eIF3f inhibits HIV mRNA 3' processing is suggested by the observation that eIF3f interacts directly with CDK11, both *in vitro* and *in vivo*. CDK11 also interacts directly with the SR protein 9G8, both *in vivo* and *in vitro*. 9G8 specifically interacts with the mammalian poly(A) site recognition factor CFI_m, and has been shown to promote mRNA 3' processing both *in vivo* and *in vitro*. We found that not only does CDK11 interact with eIF3f, but we show that there is a specific interaction between eIF3f and 9G8 *in vivo*. We further demonstrate that mutation of a potential 9G8 binding site upstream of the HIV-1 poly(A) site abolishes specific RNA/protein complex formation, and reduces the cleavage efficiency of the HIV-1 poly(A) site *in vitro*.

Taken together, a network of physical and functional interactions has been established that suggest a mechanism for the specific inhibition of HIV mRNA 3' processing by eIF3f. This mechanism involves the interaction of eIF3f with CDK11 and 9G8 and the consequent alteration of the ability of 9G8 to participate in HIV-1 pre-mRNA 3' processing.