

Discovery and analysis of cellular elongation factors that are critical for HIV-1 reverse transcription

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Using protein fractionation combined with an in vitro endogenous reverse transcription assay and mass spectrometry, we identified a cellular protein complex composed of subunits of eukaryotic elongation factor 1 (eEF1) that was able to stimulate late steps of HIV-1 reverse transcription in vitro. Further studies showed that the eEF1A and eEF1G subunits of eEF1 are important components of the HIV-1 reverse transcription complex (RTC) as evidenced by: (a) co-localization of eEF1G and eEF1A with reverse transcriptase (RT) in HIV-1 infected cells; (b) co-purification eEF1 subunits with RTC isolated from infected cells; (c) markedly reduced HIV-1 reverse transcription when eEF1A or eEF1G were down regulated by siRNA. Protein- protein interaction between recombinant RT and eEF1 complex subunits isolated from HEK293T cells was investigated by surface plasmon resonance. Both RT51 and 66 subunits were able to bind to eEF1A with a K_d of 5.4 nM and 1.9 nM, but not to eEF1B, eEF1D or eEF1G. Recent experiments indicate that eEF1A is a major RT binding protein and most likely a mediator of eEF1A complex and HIV RTC interaction as knock-down of eEF1A expression in cells by siRNA treatment resulted in significantly reduced binding of HIV RT to proteins in the cell lysate. Binding to RT could be restored by over-expression of exogenous eEF1A by plasmid transfection. The interaction of RT and eEF1A are refined by mapping the binding domains within the two proteins. The role of RT-eEF1A interaction in HIV reverse transcription represents a potential new target for anti-HIV drug screening.

Biography

David is an Australian Research Council Future Fellow and Group Leader of Molecular Virology at the Queensland Institute of Medical Research. After completing a PhD at UCLA in 1994 in Experimental Pathology of HIV-1 with Prof. Richard Gaynor, he undertook post-doctoral studies at the University of Texas Southwestern Medical Centre under an NIH Fellowship in immunology and virology. He moved to Australia in 1997 to lead a lab in the National Centre of HIV Virology Research investigating HIV reverse transcription. He has published 50 peer-reviewed papers and serves on the Editorial board of several leading journals including PLoS ONE.

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