

# The interaction of eEF1A and HIV RT is critical for HIV-1 reverse transcription and a potential anti-HIV target

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## Eukaryotic elongation factor 1 complex subunits are critical HIV-1 reverse transcription cofactors

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Cellular proteins have been implicated as important for HIV-1 reverse transcription, but whether any are reverse transcription cofactors (RTC) cofactors or affect reverse transcription indirectly is unclear. Here we used various biochemical methods to

Attempts to purify and to identify cellular RTC cofactors by more conventional methods have only partly succeeded (4, 6). Narayan et al. showed that in vitro studies of avian sarcoma and leukosis virus reverse transcriptase revealed the existence of cofactors.

- The study demonstrated that eEF1 complex is associated with HIV reverse transcription complex (RTC) and the association is critical for efficient reverse transcription.

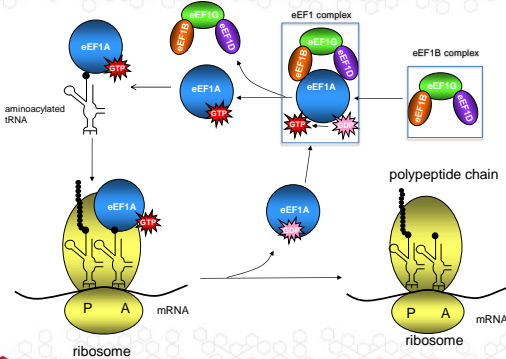


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## eEF1 complex and the role in translation elongation



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## Aims of the study

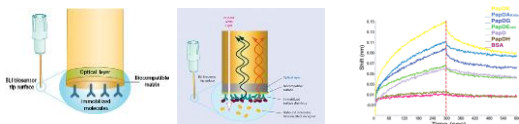
1. Characterize the association between reverse transcriptase complex (RTC) and eEF1 complex.
2. Assess the possibility the association can be a potential anti-HIV target.



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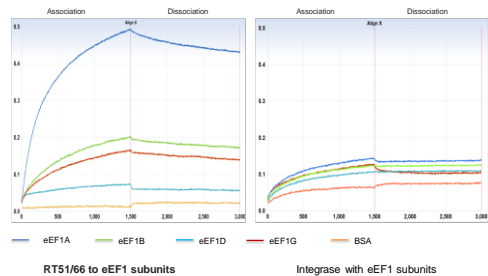
## Dissection analysis of the association of RTC and eEF1 complex

Bio-Layer Interferometry (BLI) assays using Octet system



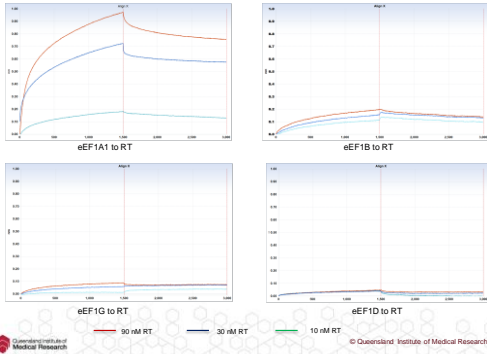
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## HIV RT interacts with eEF1A, not any other factor



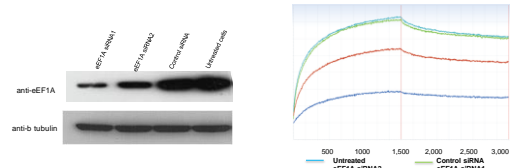
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**Interaction of eEF1A and RT was confirmed by reverse BLI assay**



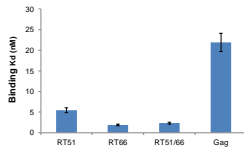
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**eEF1A is a predominant RT cellular binding protein**

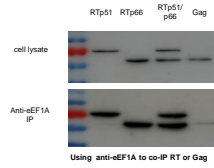


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**Binding affinity of eEF1A to RT and Gag proteins measured by BLI assay**

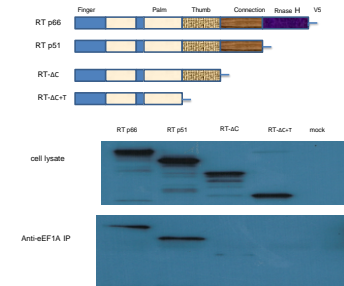


**Interaction of eEF1A with RT was confirmed by CO-immunoprecipitation**



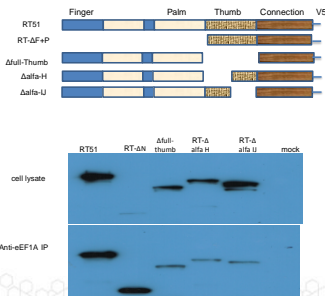
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**C-terminus of RT is the region for eEF1A interaction**



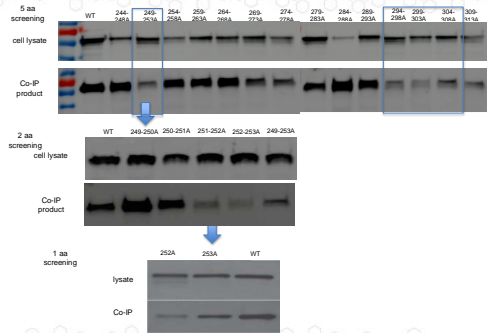
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**RT thumb and connection domains are essential and sufficiently for eEF1A interaction**



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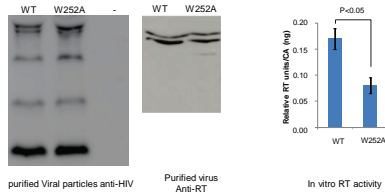
**Screening RT thumb domain by alanine substitution for 1A1 interaction**



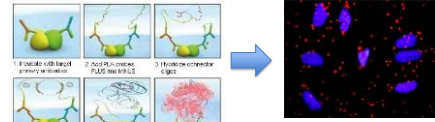
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**The RT from RT W252A mutated virus remains intact, but the activity is reduced in the *in vitro* assay**

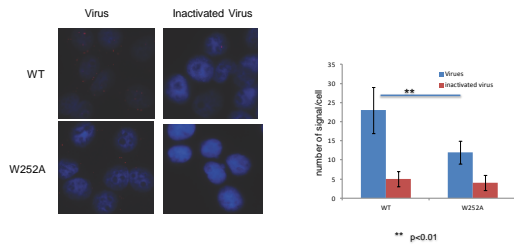
W252A mutation was introduced into pGCHIV-1-NL4-3



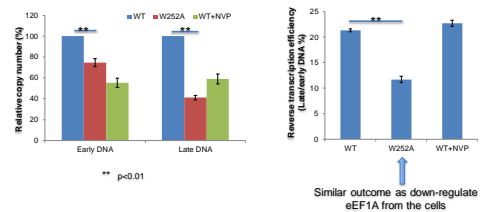
**Duolink Proximity Ligation Assay (PLA) was used to examine changes of eEF1A and RT co-localization in infected cells**



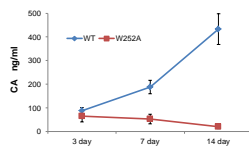
**RT W252A mutation results in reduced eEF1A-RT co-localization in infected cells**



**Virus containing RT W252A mutation has reduced reverse transcription efficiency**



**Virus containing RT W252A mutation lost infectivity**



**Discussions**

**Why eEF1A is important?**

Properties and multiple cellular roles that may be important for virus replication:

- Ability to bind with both RNA and protein, properties of protein chaperone.
- A machinery of Nucleo-cytoplasmic trafficking.
- multiple aspects of cytoskeletal regulation.
- Protein degradation and cellular apoptosis

## Discussions

eEF1A involves in multiple stages of RNA virus replication for viruses from various families

- Interacts with virus polymerase, play roles in formation and stability of replication complex (tobacco mosaic virus, turnip mosaic virus, VSV, West Nile Virus).
- Interacts with viral structural proteins and facilitates virus particle assembling (VSV, vaccinia virus, CMV, SARS-CoV and HIV-1).
- Interacts with various virus genomic RNAs, on RNA-structure based manner, to regulate translation and transcription (turnip yellow mosaic virus, tobacco mosaic virus, brome mosaic virus, West Nile virus, Hepatitis delta virus, poliovirus).



## Conclusions

- eEF1A-RT interaction mediates the association of RTC and eEF1 complex.
- The interaction of eEF1A-RT is crucial for intact RT production and virus replication.
- The tight and specific interaction between eEF1A and RT can be a potential anti-HIV target, which will be discussed by David Harrich in the afternoon session.



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