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A qPCR assay for measuring the post-integrational DNA repair in HIV-1 replication

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Highlights:

- A qPCR assay to measure the DNA repair efficiency in HIV-1 replication is developed
- Post-integrational DNA repair process takes 8-9 hours post integration
- Inhibition of DNA-PKcs by Nu7441 decreases post-integrational DNA repair efficiency

Abstract

The post-integrational gap repair is a critical and poorly studied stage of the lentiviral life cycle. It might be performed by various cellular DNA repair pathways but the exact mechanism of the repair process has not yet been described. One of the reasons for that is the lack of a functional quantitative assay that could precisely measure the amount of integrated viral DNA that has completed the post-integrational gap repair stage. Here, we present an approach that is based on a widely used Alu-specific PCR for the estimation of integrated viral DNA but includes several steps that allow discrimination between integrated-repaired and integrated-unrepaired viral DNA forms. We used the approach for the estimation of the kinetics of gap repair in a viral vector system and showed that the gap repair process starts at 17 hours post infection and lasts 10 more hours. We also showed that the addition of Nu7441 – a small molecule inhibitor of DNA-breaks sensor kinase in the non-homologous end joining DNA repair pathway - specifically inhibits the gap repair process while having no influence on the integration itself.

1. Introduction

The early stages of HIV-1 (Order *Ortervirales*, Family *Retroviridae*, Subfamily *Orthoretroviridae*, species *Human immunodeficiency virus type 1*) infection involve the entry of the viral core into the cell cytoplasm, reverse transcription resulting in the formation of a linear double stranded DNA copy of the viral genomic RNA, and integration of the viral DNA