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A Kinetic View of Viral RNA in Single Live Cells in Real Time

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Abstract

Viral RNA biogenesis is a crucial step in the replication of RNA viruses and retroviruses that require both the production of genomic RNAs and of translation templates. Cellular and viral factors concur in the biogenesis of RNA at the specific sub-cellular site where the reaction takes place. The possibility of tracking viral RNA in living cells gives the unique possibility of measuring the kinetic parameters of RNA biogenesis as well as defining the dynamic recruitment of host and viral factors to the site of replication.

By engineering a RNA-tagged human immunodeficiency (HIV-1) retrovirus we could characterize the HIV-1 transcription cycle allowing precise kinetic measurements of RNA polymerase elongation rates as well as initiation, splicing and termination steps (Boireau, et al. JCB 2007). In addition we also analyzed the dynamic of the TAR:Tat:pTEFb complex at the site of HIV-1 transcription in living cells (Molle, et al. Retrovirology 2007). Our data suggest that this complex dissociates from the polymerase following transcription initiation, and may undergo subsequent cycles of association/dissociation.

We extended this approach to the tick-borne encephalitis virus (TBEV). Flaviviruses are positive RNA viruses that assemble the replication complex in the cytoplasm of the infected cells (Miorin, et al. 2008). The modified TBEV replicons were competent for RNA replication and allowed the visualization of replicated genomic RNA that accumulated in cytoplasmic structures with a distinct sub-cellular localization. A dynamic view of TBEV RNA biogenesis in living cells can be seen at ftp://ftp.icgeb.org/pub/tmp/BHKA3movie.avi.

This work provides for the first time a kinetic framework to analyze viral RNA biogenesis and RNA/protein dynamics in living cells.