INCORPORATION OF FLUORESCENT NUCLEOTIDES AS A LABELER TO HUMAN RHINOVIRUS VIRAL RNA

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INTRO: Specific localization of RNAs in cells is an important mechanism to control protein expression and function. Labeling specific RNA molecules inside live cells is one of the biggest challenges in the investigation of RNA translocation. Investigating viral RNA translocation poses an additional difficulty because of the small amount of viral material inside the cells in the beginning of the infection. The use of radioactive uridine or bromus-labeled uridine has been described as tools to nonspecifically tag RNA strands and determine their distributions in different types of cells, including infected cells. 

We propose a new strategy to label specific viral RNA taking advantage of the selective binding of RNA by viral capsid proteins. Incubation of infected cells with BrU during the viral replication step results in the assembly of new infectious RNA strands. It is known also that virus packages RNA selectively. Therefore, it is possible to produce BrU-labeled virions by offering BrU to the virus to produce new RNA strands during infection.

OBJECTIVE: To follow the viral RNA pathway translocation using BrU, in order to investigate the packaging of viral RNA dynamics during infection.

METHOD: Hela-H1 cells were infected with Human Rhinovirus, type B, serotype 14 (HRV-B14). HRV belongs to the Picomavirus family and Rhinovirus genus, and it is the main causative of common cold, and incubated with BrU 2.5 mM for 20h. New progeny containing BrU-RNA was purified and confirmed by Western Blotting the HRV particle BrU label and we used immunocytochemistry labelling of new infected cells to confirm the delivery of BrU labelled viral RNA in different steps of the infection process the beginning of infection.

RESULTS AND DISCUSSION: Initial results showed that BrU was delivered to the cells successfully in the beginning of infection. We also observed that BrU...
did not affect significantly viral replication or cellular viability. On the other hand, the results did not showed if the BrU was incorporated to HRV after cell infection.

CONCLUSION: The visualization of viral nucleic acid inside the cell is a rough task, and very difficult to accompany follow the viral genetic material nucleic acid inside the cell during the infection. BrU-labeling of nascent particles method is a promising approach to clarify illustrate the process some points of viral RNA translocation during infection. Even though, other tests are necessary to evaluate the efficiency of BrU as a viral RNA labeler.

KEYWORDS: Bromouridine, RNA packaging, HRV.

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