



Development and use of a kinetical and real-time monitoring system to analyze the replication of hepatitis C virus

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論文題目

Development and use of a kinetical and real-time monitoring system to analyze the replication of hepatitis C virus

(C型肝炎ウイルスの複製を解析するための動態学的かつリアルタイムモニタリングシステムの開発と利用)

論文の内容の要旨

[Introduction]

In microbiological research, it is essential to understand the time course of each step in the lifecycle of a pathogen and how the host cell environment changes over time after infection. However, analyses of temporal changes in the lifecycles of viruses and other pathogens have been performed based on endpoint assays in which materials including cell culture supernatants and lysates are collected over time and analyzed for viral gene products or reporter activities. This study developed for the first time a real-time recording system of replication of hepatitis C virus (HCV) through monitoring reporter activities using a photomultiplier, which is a highly sensitive luminescence detector, capable of continuously detecting weak luminescence over time in culture settings without sampling.

[Materials and Methods]

A subgenomic HCV replicon plasmid pSGR-JFH1/WT/SLG, which carries the viral cDNAs of 5'- and 3' non-coding regions and non-structural NS3-NS5B regions derived from HCV JFH-1 strain as well as the luciferase SLG gene as a reporter, was constructed by substitution of a fragment containing SLG from pSLG-HSVtk (Toyobo) into pSGR-JFH1/FLuc. Replicon RNAs were synthesized *in vitro* by a MEGAscript T7 kit (Ambion) using linearized DNA fragments as templates. Human hepatoma HuH-7 derived cells where replicon RNAs were introduced were cultured with media containing D-luciferin as a substrate for SLG and luminescence signals in the culture media were recorded for 1 min at intervals of 29 min using Kronos Dio Luminometer (ATTO). The measurement was continued without exchanging the culture medium.

[Results]

The kinetically-monitored bioluminescence patterns indicating RNA replication of HCV were in good agreement with the temporal changes in NS5A protein expression in cells carrying the viral replicon RNA. A comparison of bioluminescence profiles from the wild-type and polymerase-defective-mutated replicons, in combination with the patterns seen upon the addition of antivirals, indicated that each signal peak represents translation and replication processes, respectively.

Time-course analyses showed that transient translation and genome replication can be detected separately, with the first peak of translation observed at 3-4 h after introduction of the viral RNA into cells and replication beginning around 20 h after viral RNA introduction into cells. From the bioluminescence data set measured every 30 min (48 measurements per day) for 10 days, the initial velocities of translation and replication were calculated, and their capacity levels were expressed as the sums of the measured signals in each process, which correspond to the areas on the kinetics graphs.

Comparison of various HuH-7-derived cell lines showed that the time from RNA introduction to the start of RNA replication and the initial velocity of replication were shown to be diverse among the HuH-7 subclones, and that the cell lines with higher replication capacity tended to have a shorter time to the start of replication and a higher initial velocity of replication.

The effects of RNA mutations within the 5' UTR of the replicon on viral translation and replication were further analyzed in the system developed, indicating that mutations to the miR-122 binding sites primarily reduce replication activity rather than translation.

[Discussion]

The real-time monitoring system established using HCV replicon as a model enabled us to conduct detailed time-course analyses of viral lifecycles, such as how their processes are connected and transitioned. Based on the findings obtained, since HCV RNA replication initiates after a certain interval of time after transient translation reaches its peak, it is likely that not only the production of viral proteins but also the development of an intracellular environment suitable for viral replication are required for replication initiation. Maturation of viral non-structural proteins and their interaction with host-cell factors presumably at the endoplasmic reticulum-derived membrane may reflect the time at which the scaffold for replication and the replication complex are formed. Since the difference in translation efficiency among the four cell lines used was not as much as the difference in replication efficiency, the differences in time to replication initiation and replication velocity may not be due to differences in the expression level of the viral proteins, but rather to providing an intracellular environment suitable for viral replication.

[Conclusion]

This is the first study to develop a system for real-time recording of luciferase activity as an indicator of viral replication without the need to collect replicating cells over time. The system requires no extra intervention by the experimenter for data collection, minimizes artifacts generated during cell manipulation and sample preparation, and accurately performs continuous multiple measurements. Taking advantage of this feature, this system should be useful not only for viral replication analysis, but also for

analysis of the entire infection cycle of various viruses, including the early stages of viral infection, and for analysis of temporal environmental changes on the host cell side associated with infection.