INFLUENCE OF THE CELL CYCLE ON THE INFECTIOUS TITER OF MURINE HEPATITIS VIRUS, STRAIN A59

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Several studies have emphasized the importance of the cell growth phase in the gene expression of retroviruses and the polyomavirus SV40. On the other hand, scarce studies have correlated the efficiency of replication of other viruses with the cell cycle. Joseph et al. (1975) showed a 10,000-fold increase in Measles virus production after mitogenic stimulation of acutely infected lymphocytes. Ogura et al. (1984) correlated Sendai virus production in persistently infected cells with the cell growth phase. However, the observation only stemmed from a comparison of resting or actively growing cells. The same approach was used by Pons et al. (1983), who showed a 20- to 60-fold increase in respiratory syncytial virus production in actively growing HEp-2 cells.

In the course of our studies on murine hepatitis virus (MHV), we also observed increased virus titers after infection of actively growing DBT cells. In order to more precisely define the importance of the cell cycle in the replication of the A59 strain of murine hepatitis virus (MHV-A59), we synchronized DBT cells at the G1/S boundary of the cell cycle. This was achieved by adding the drug aphidicolin, an inhibitor of DNA polymerase α (Pedrali-Noy et al., 1980), at a final concentration of 5 µg/ml for 24 hrs. The drug was removed for 9 hrs and readded for a final 12 hrs in order to accumulate non-synchronized cells at the boundary between the G1 and S phases.

The DBT cell cycle was determined to last a total of 12 hrs, with a 6-hr S phase, using 20-min tritiated thymidine pulses and total cell counts at various times after removal of the aphidicolin and thus release from the G1/S block (Volpe and Eremendo-Volpe, 1970).

Synchronized DBT cells were infected with twice plaque-purified MHV-A59 at a multiplicity of infection of 0.01 at different stages of the cell cycle. The infectious titer of progeny virions accumulated in the medium for 14 hrs was then measured by plaque assay on DBT cells. Control non-synchronized cells were similarly infected. Parallel cultures were used for cell counts in order to determine the output of infectious virus per cell.

M. M. C. Lai et al. (eds.), *Coronaviruses*

Table 1 - SUMMARY OF VIRUS TITERS OBTAINED AFTER INFECTION AT VARIOUS STAGES OF THE CELL CYCLE

Time after release from G1/S block (hrs)	Phase of the cell cycle	Infectious virus titer PFU/ml ($\times 10^{-7}$)	PFU/cell ($\times 10^{-2}$)
0	S	2.2	2.4
2	S	12.0	13.3
4	S	6.0	6.7
6	S	3.0	2.0
8	G2/M/G1	1.1	0.73
10	G2/M/G1	7.0	3.5
12	G2/M/G1	5.6	2.8
14	S	12.0	6.0
16	S	3.8	1.9
18	S	14.0	7.0
Non-synchronized		2.0	2.2

Analysis of results presented in Table 1 suggests a 6- to 12-fold increase in the efficiency of DBT cell infection by MHV-A59 in the early stages of the S phase of the cell cycle, as monitored by infectious titers of progeny virions released in the culture medium. A more efficient viral infection in this active phase of cell growth probably reflects the importance of the cellular biosynthetic machinery in viral replication. Previous studies have shown that MHV-A59 is quite inefficient at shutting-off host synthetic processes (Rottier et al., 1981), most likely because they are needed for maximum efficiency of its own replication. The less striking correlation observed during the second cell cycle could probably be explained by a gradual loss of cell synchrony.

Such a dependence on the cell cycle for coronavirus replication may help explain the various levels of viral replication observed in individual cells in a non-synchronized population. Moreover, cell synchronization, easily achieved with aphidicolin, provides a simple approach to boost infectious titers.

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Supported by National Sciences and Engineering Research Council of Canada (Grant U0387 and Scholarship to P.J.T.).