Abstract

Mouse polyomavirus is a type species of Polyomaviridae family and serves as model for studying viral infection of human pathogenic polyomaviruses. Minor proteins of viral capsid have been found to be necessary for effective initiation of infection. In order to study their role in the early steps of infection we utilized the novel Cre-LoxP system for production of the viral mutant lacking both minor proteins. Virus produced this way was compared with virus produced by standard method and we found that both systems facilitate production of mutant virus with the comparable quality and quantity. The mutant virus contained reduced amount of viral DNA and formed virions with impaired stability. For further studies of intracellular virion trafficking we prepared virions with genomes modified by thymidine analogues 5-bromo-2'-deoxyuridine (BrdU) and 5-Ethynyl-2'-deoxyuridine (EdU) and optimized the methods for analogue detection. The viral genome become accessible for detection 4 hours post infection. For ultramicroscopic analysis of translocation of virus to the nucleus we used freeze substitution. All this methods will be utilized for detailed study of distinct steps in viral infection.

Key words: Mouse polyomavirus, minor proteins, mutant virus, Cre recombinase, BrdU, EdU, Click Chemistry