The Interrelationship Between the Replication And Maturation Pathways of SV40

by

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ABSTRACT

Newly synthesized simian virus 40 (SV40) DNA is used as a template for further DNA synthesis (reenters replication) or as a substrate in the assembly of virions (maturation pathway). The factors determining the fate of newly synthesized DNA are poorly understood. Previous work has shown that this DNA remains available to be used in the replication pathway for a limited period of time. Experiments were, therefore, designed to determine if its removal from the replication pathway correlated with its appearance in the maturation pathway. The time courses of reentry into the replication pathway and progression along the maturation pathway were both determined on identical samples or in parallel cultures isolated from cells infected with wild type SV40. DNA synthesized during a 20 min pulse reentered replication over a period of several hours and then was removed from the pool of molecules available for replication. The cessation of reentry coincided with the maturation of this DNA from chromatin to previrions and virions. More reentry and less maturation was observed at 24 h post infection than at 42 h post infection.

Since cessation of reentry was temporally linked to progression into the maturation pathway, the effect of deletions located in the region coding for structural proteins on these two pathways was analyzed. Deletion mutant and wild type DNAs were obtained using recombinant DNA techniques. The DNAs were introduced into cells using the DNA transfection method. The DNAs from two deletion mutants, dlBE and dl1133, had reentry kinetics similar to wild type. Two others, however, dl1007 and dl1003, continued to reenter replication after wild

type DNA had ceased to reenter. When cells were cotransfected with mutant and wild type DNA, the mutant DNA remained in the replication pathway for a greater period of time. These results strongly suggest that there is a cis-acting element which promotes efficient removal of DNA from the replication pathway. This cis-acting DNA sequence is located within the Hind III E fragment, the region common to the VP2 and VP3 coding sequence. DNA from deletion mutants lacking this sequence did not progress from chromatin to previrions and virions. In the presence of wild-type, the encapsidation efficiency of these DNAs was lower than that seen with wild type DNA.

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