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Hitchhiking a Big Band: A Novel Tactic for the Stable Maintenance of Herpesviral Genome

Chunghun Lim[PI], Joonho Choe[CI]

Department of Biological Sciences, KAIST, Daejeon 305-701

The maintenance of viral genome in infected cells is essential for the survival of infecting viruses. Otherwise, they may be diluted out during successive cell divisions of host cells. DNA viruses, which possess extrachromosomal genome in infected cells, have evolved strategically conserved but tactically different mechanisms for the persistence of viral genome. Generally, it is accomplished by two distinct but intimate events. These are the DNA synthesis of viral genome and the equal segregation of replicated genome into two daughter cells. In the case of papillomavirus, a viral helicase E1, in concert with a viral auxiliary protein E2, unwinds viral origin of replication and initiates DNA synthesis from the cis-element of papillomavirus replication. However, long-term maintenance of viral oriP-containing plasmid requires additional viral cis-element designated as a minichromosome maintenance element (MME). MME is located within long control region (LCR), distinct from the replication origin, and contains multiple high-affinity E2 binding sites. Accumulating data suggest that chromatin-bound E2 tethers viral genome to host chromosome via this MME, thereby permitting the equal segregation and nuclear retention of replicated viral genome during mitosis. In the case of EBV, two viral cis-elements constitute origin of replication for the latent replication of EBV. These are family of repeat (FR) and dyad symmetry (DS) sequences, which are separated by ~ 1 kb within EBV genome. Both sequences contain binding sites for EBNA-1, a viral trans-element for the latent replication of EBV. In contrast to papillomavirus E1, EBNA-1 does not have any apparent enzymatic activity for DNA replication. However, recent reports suggest that EBNA-1 recruits pre-replicative complex to viral oriP for the replication. DNA synthesis initiates from DS containing low-affinity EBNA-1-binding sites and DS is necessary and sufficient for the transient replication mediated by EBNA-1. However, the stable maintenance of EBV oriP-containing plasmid additionally requires FR containing multiple high-affinity EBNA-1 binding sites. As in the case of papillomavirus E2, EBNA-1 associates with host chromosomes and it has been suggested that the plasmid retention activity of FR would involve the hitchhike of viral genome to host chromosome through EBNA-1. Kapos i' s sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi' s sarcoma and several lymphoproliferative diseases including primary effusion lymphoma, also called body-cavity based lymphoma, and some cases of multicentric Castleman' s disease. The latency-associated nuclear antigen (LANA) of KSHV has been implicated in the maintenance of the viral genome during latent infection. The C-terminal DNA-binding domain of LANA interacts with sequences located in terminal repeats (TRs) of viral genome, and the N-terminal chromosome-binding sequence (CBS) of LANA associates with host chromosomes. LANA co-localizes with KSHV genome on the host chromosome, suggesting that LANA tethers viral genome to host chromosome for its persistence in infected cells. This chromosome-tethering model seems to be conserved among DNA viruses such as papillomavirus and Epstein-Barr virus (EBV), which possess extra-chromosomal genome during their latent infection. Using a long-term replication assay, it has been previously shown that KSHV TR and LANA act as cis- and trans-elements for the persistence of viral genome, respectively. In this study, we established a transient replication assay with a methylation-sensitive restriction enzyme, DpnI, and confirmed that LANA also actively participates in the replication of TR-containing plasmid. Using this assay system, we found that 293, 293T, BJAB, C33A, HCT116, and COS-1, but not NIH/3T3 cell lines are permissive for the replication of KSHV TR-containing plasmid by LANA, and further characterized viral cis- and trans-acting elements of KSHV latent replication. Transient reporter assay and transient replication assay disclosed that the orientation and basal transcriptional activity of TR constructs did not significantly affect the efficiency of replication. However, at least two TR units were necessary for efficient replication. The N-terminal 22 amino acids comprising the CBS of LANA were necessary and sufficient for the mediation of its C-terminal DNA-binding and dimerization domain to support the transient replication of KSHV TR-containing plasmid, although the C-terminus alone can bind sequences within TR and repress the TR-dependent transcription in electrophoretic mobility shift assay and transient reporter assay, respectively. We generated several point mutations on sequences within LANA CBS and tested their functional activities including sub-cellular localization, mitotic chromosome association, biochemical fractionation, inhibition of TR-dependent transcription, and transient replication of KSHV TR-containing plasmid. Our data indicate that chromosome-binding activity of LANA is prerequisite to its replication activity and the latent DNA replication of KSHV genome seems to be tightly coupled to its equal segregation and nuclear retention, while papillomavirus and EBV have evolved independent mechanisms for DNA replication and partition of viral genome.