Herpes Simplex Virus 1 DNA replication and its role in recombination and transcription

Akademisk avhandling

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av

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Avhandlingen är baserad på följande arbeten:

I. Stepwise evolution of the herpes simplex virus origin binding protein and origin of replication.
Olsson M, Tang KW, Persson C, Wilhelmsson LM, Billeter M, Elias P.
J Biol Chem. 2009 Jun 12;284(24):16246-55.

II. Rad51 and Rad52 are involved in homologous recombination of replicating herpes simplex virus DNA.
Tang KW, Norberg P, Holmudden M, Elias P, Liljeqvist JÅ.
Plos One. 2014 Nov 3;9(11):e111584.

III. Replication-dependent expression of herpes simplex virus 1 late genes is controlled by P-TEFb and DSIF.
Tang KW, Zhao ZY, Samuelsson T, Elias P.
Manuscript

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Herpes simplex virus 1 (HSV-1) is one of nine different herpesvirus infecting man. They are all capable of establishing a life-long latent state following the primary infection. HSV-1 as well as other herpesviruses may reactivate from the latent state and give rise to a productive infection with clinical symptoms or asymptomatic shedding. HSV-1 infections are primarily treated by targeting the viral DNA replication carried out by a molecular machinery, a replisome, encoded by the virus. Here we examine the mechanism of initiation of viral DNA replication and also how viral DNA replication interacts with DNA recombination and gene expression.

In our first study we examined the initiation-step of HSV-1 replication. The origin binding protein (OBP) initiates replication by binding to the origin of replication (oriS and/or oriL). We showed, using phylogenetics and biochemical experiments, that there was a step-wise evolutionary development of herpesvirus DNA replication initiation. The initial divergence was seen in herpesviruses acquiring an amino-acid motif RVKNL in OBP which binds the sequence TTCGCAC in the oriS. The next step was the development of an ICP8 binding motif at the C-terminus of OBP and finally the arrangement of the binding-sites for OBP in the oriS-sequence and the ability to form a stable hairpin. We presented molecular in vitro data to support the phylogenetic analysis and thereby defining essential motifs in OBP for protein-protein and protein-DNA interactions.

The next study was focused on genetic recombination between different HSV-1 strains. We followed the propagation of HSV-1 in cells infected with one to three genotypes of HSV-1 and calculated the number of recombination events. We found evidence for Rad51 and Rad52 involvement in recombination of the unique long and unique short gene segments of HSV-1. We also observed an increased recombination rate in cells with retarded ligation of Okazaki-fragments. The fidelity of recombination in virus propagated through mixed infections appears to be high since expansion or shortening of repeated sequences in the US7 gene was not detected.

In the third study we examined the replication-coupled transcription of HSV-1 late genes, which are known to depend on DNA replication for efficient expression. Using chromatin immuno-precipitation we could determine that recruitment of RNA polymerase II to late gene promoters occur even in the absence of replication. Recruitment was dependent on ICP4, but delayed in comparison with early gene promoters. These observations suggested the involvement of transcription elongation and/or maturation in the expression of gamma genes. By using the drug DRB, which inhibits the kinase CDK9, a component of the positive transcription elongation factor B, and siRNA against Spt5, a transcription processivity factor, we could show a specific impairment of late gene expression, with only minimal effect on early gene expression and DNA synthesis. We suggest that CDK9 and Spt5 are specifically recruited to replicated late genes and mediate a maturation of transcribed mRNA for nuclear exit.

In summary, we have studied essential molecular processes in the HSV-1 life cycle and identified molecular interactions as well as mechanistic pathways, which may serve as future drug targets.

Keywords: Herpes Simplex Virus 1, DNA replication, DNA recombination, Rad51, Rad52, Transcription, CDK9, Spt5

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