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Molecular aspects on Herpes simplex virus type 1 DNA replication and gene expression

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Carl Kylberg, Medicinaregatan 7, fredagen den 26 januari 2018, klockan 13:00

av Zhiyuan Zhao

Fakultetsopponent:
Professor Erik Johansson
Umeå universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. Muylaert, I, **Zhao, Z**, Andersson, T, Elias P. Identification of Conserved Amino Acids in the Herpes Simplex Virus Type 1 UL8 Protein Required for DNA Synthesis and UL52 Primase Interaction in the Virus Replisome J Biol Chem. 2009 July; 287(40): 33142-52

II. Muylaert I, **Zhao, Z**, Elias P. UL52 Primase Interactions in the Herpes Simplex Virus 1 Helicase-Primase Are Affected by Antiviral Compounds and Mutations Causing Drug Resistance J Biol Chem. 2014 October; 289(47): 32583-92

III. **Zhao, Z***, Tang KW*, Muylaert I, Samuelsson T, Elias P. CDK9 and SPT5 proteins are specifically required for expression of herpes simplex virus 1 replication-dependent late genes J Biol Chem. 2017 July; 292(37): 15489-00

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Abstract

Herpesviruses infect a variety of animals from molluscs to humans and they have evolved in a close relationship with their hosts. In humans, we find nine herpesviruses, representing all three subfamilies of the *family Herpesviridae* and they can cause a variety of symptoms. The viruses have evolved independently, but they have all kept a conserved molecular machinery for the replication of their genomes. We have been studying the protein interactions within the molecular machinery of herpes simplex virus I (HSV-1), to gain further insight into the molecular mechanism of how the virus replicates and maintains its genome. In addition, we have been investigating the molecular requirements for the expression of the HSV-1 DNA replication-dependent late genes. We expect that detailed knowledge of these molecular events will help the development of new antiviral therapies, and perhaps also promote the understanding of related events in our own cells.

In this thesis, we have shown that the interactions between the seven viral proteins, which are essential for the HSV-1 DNA replication, are species-specific. The proteins cannot be substituted with homologs from a closely related virus, Equine herpesvirus 1. This observation suggests that the seven replication proteins function as a molecular machinery unit, a replisome, which is characterized by numerous protein-protein interactions. Additionally, we have identified important amino acids in an enzymatically inactive protein, UL8, in the HSV-1 helicase-primase complex, which is required for its interaction with the primase component, UL52, in the complex. Mutations of these amino acids in UL8 impaired their interaction and reduced or abolished the DNA replication capacity of the HSV-1 replisome at the non-permissive temperature.

Next, we examined the interactions between UL52 of the HSV-1 helicase-primase complex and other replication proteins. We found that UL52 consisted of different domains, and that the domains had different interaction partners. Stable interactions were detected between the N-terminal domain of UL52 and the helicase component, UL5, while the middle domain showed stable interactions with UL8. We could only detect a relative weak association between UL5 and the C-terminal domain of UL52, which may suggest the existence of a transient interaction. Furthermore, a new group of drugs against HSV infection targets the helicase-primase complex, but their inhibitory action was unknown. We have now demonstrated that these drugs inhibit HSV-1 DNA replication by affecting the interaction between UL5 and UL52. We suggest that the drugs lock these proteins in a certain conformation, preventing them from assisting in viral DNA replication.

In addition to its interaction within the helicase-primase complex, the middle domain of UL52 also exhibited stable interaction with HSV-1 single-strand DNA binding protein, UL29/ICP8. The interaction between these two proteins may indicate how the helicase-primase complex is loaded onto the activated origins of replication in the HSV-1 genome, and synthesizes primers on single-stranded DNA coated with ICP8.

Viral DNA replication is a prerequisite for the expression of HSV-1 true late genes. We have shown that expression of true late genes is also specifically dependent on the activity of CDK9, a cellular protein kinase involved in transcriptional regulation. The inhibition of CDK9 affected the transcription and cytosolic accumulation of viral late mRNAs. Furthermore, a substrate of CDK9, the transcription factor SPT5, was shown to be necessary for late gene expression. The activity of CDK9 was necessary for an interaction between SPT5 and the viral protein ICP27, which is suggested to be involved with the maturation and nuclear export of viral late mRNAs. Our results suggest that the control of HSV-1 true late genes are at least partially regulated by the maturation and nuclear export of viral late mRNAs.

Keywords: Herpes simplex virus type 1, DNA replication, gene expression

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