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Molecular Modification of a HSV- 1 Protein and Its Associated Gene Transcriptional Regulation*

Yan-chun CHE，Li JIANG and Qi-han LI**

(Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 379 Jiao Ling Road, Kunming 650118, China)

Abstract: The molecular modifications of Herpes Simplex Virus Type I （HSV-1） proteins represented by acetylation and phosphorylation are essential to its biological functions. The cellular chromatin-remodeling/ assembly is involved in HSV-1 associated gene transcriptional regulation in human cells harboring HSV-1 lytic or latent infections. Further investigation on these biological events would provide a better understanding of the mechanisms of HSV-1 viral gene transcriptional regulation.

Key words: Modification; Herpes simplex virus type (HSV-1); Transcriptional regulation

There has been extensive investigation of the molecular biology of Herpesviruses, and how to understand the specific infectious pathways of the Herpesvirus, including co-establishment of lytic and latent infection in human cell from the point view of virus to cell, has become a major interest in the field of Herpesviruses research (17). The herpes simplex virus type I (HSV-1) has the capacity to generate a transcriptional regulatory system that can drive its propagation in infected cells via interactions with associated cellular molecules and its own virally encoded proteins (11). This regulatory system and its interactions with the host can determine the outcome of infection, resulting in either a lytic or a latent outcome. Some viral proteins are likely to act directly on cellular proteins involved in the regulation of gene transcription, as shown in an epigenetic model (22). Such findings have demonstrated the functional diversity of HSV-1-associated elements in the infectious process and viral replication.

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** Corresponding author.
Tel.:+86-871-8334554; Fax: +86-871-8334483
E-mail: Imbcams.LQ@gmail.com

HSV-1 PROTEIN MODIFICATION EFFECTS

VP16 is a well-studied viral capsid protein that is known to play a major role in the activation of HSV-1 gene transcription. By binding to cellular transcriptional factors such as the host cell factor (HCF) and octamer-binding protein 1 (Oct-1) (27), it initiates the HSV-1 immediately-early (IE) gene transcriptional process and has now become a typical model of viral gene transcriptional activation (21). However, increasing numbers of studies indicate that VP16 is frequently linked to many protein modifications with functions that are not yet completely defined. The transcriptional activating structural domain (AD) of VP16 is also capable of

interacting with several cellular histone acetyltransferases (HATs) such as P300 and the CERB-binding protein (CBP) (12). These transcriptional regulatory auxiliary factors are believed to be involved in chromatin-formed gene transcription (24). The AD domain of VP16 is also able to interact with the ATP-dependent chromatin-remodeling complex BRM and BRG1 in mammalian cells (10). Further analysis of these interactions suggest that VP16 is likely to transcribe the HAV-1 α gene via binding to HCF and Oct-1 by recruiting transcriptional regulatory auxiliary factors associated with chromatin-remodeling/assembly (10). Whereas the study of the recombinant virus reveals an obviously increased level of histone H3 on the HSV-1 α gene promoter, and in contrast an obviously decreased level of acetylation histone on the β gene promoter in the absence of VP16 (20).

Taking all of these data together, it is proposed that use of the acetylation modification enzyme system in accomplishing chromatin transcriptional regulation might contribute to the transcriptional activation function of VP16 in virus to cell infection. However, whether acetylation of the VP16 molecule modified takes place in such a process remains unknown. The VP22 capsid protein has the potential to modify the acetylation of VP16 which could subsequently affect viral gene transcriptional efficiencies resulting from its interaction with VP16 (28).

ICP0 is another well-recognized transcriptional regulatory factor. ICP0 can interact with cellular modification enzymes and appears to link to the cellular acetylation/deacetylation system with the viral α and β gene transcriptional regulation efforts. It has been reported that viral mutants with an ICP0 deficiency have the capacity to recover its infectious efficiency when histone deaceytlase (HDAC) inhibitor is used (8). Furthermore, as shown in an immunoprecipitation assay, ICP0 can form complexes with HDAC 5, HDAC 6, and HDAC 7 in ICP0-transfected cells (4). Other studies demonstrate that ICP0 exhibits a functional physical interaction in yeast-two-hybridization assays with PCAF and contributes to the enhanced acetylase activity of the p300/CBP associated factor (PCAF). These activities are likely to result in a significant increase in transcriptional activation of HSV-1 early TK or late gC gene promoter in a non-infectious CAT reporter gene system (15). In spite of a lack of supporting evidence, this model would suggest that ICP0 is in fact involved in the coordination of associated elements in the cellular acetylation/deacetylation system in addition to acting as an essential regulatory factor in HSV-1 early or late gene expression. These virus-encoded enzymes comprise the majority of molecules in this highly sophisticated transcriptional regulatory system. They specifically target cellular chromatin-remodeling/assembly pathways, significantly affect cellular differentiation and development, and could play a substantial role in the co-evolution of the virus and its host (23).

Like the ICP4, ICP22 and ICP27 IE proteins, ICP0 is phosphorylated after the association of viral or cellular enzymes (1). This modification might produce an important change in its molecular biological characterization and function. Mutation of some phosphorylation sites in ICP0 is reported to cause alteration of its localization in cells (5). ICP0 is usually localized in the ND10 domain in the nucleus of HSV-1 infected cells with its identified structural complexes including more than 10 kinds of associated molecules directly related to cellular transcriptional

regulation of PML, CBP, SP100, and Daxx (7). The localization of ICP0 in the ND10 structural domain contributes to the infectious process by affecting the recruitment of different transcriptional factors with different regulatory pathways in cells. The localization of ICP0 and its ability to recruit various molecules are directly associated with its phosphorylation. The alteration of ICP0 phosphorylation is likely to lead to decreasing transcriptional activity at HSV-1 early and late gene promoters (3). This idea is supported by similar observations in other IE proteins. For instance, ICP27 can stimulate the redistribution of CK2, a highly important cellular protein kinase in cytoplasma and nuclei during the early stages of HSV-1 infection. ICP27, during its interaction with CK2, can be phosphorylated and interact with several elements such as P32 and Alu/REF in pre-mRNA splicing. Additionally, ICP22 appears to cause the abnormal phosphorylation of the heterogeneous nuclear ribonucleoprotein K (hnRNPK) associated with CK2 pre-mRNA transport resulting in alteration of normal pre-mRNA transport functions (26).

Further investigation into the mechanisms of this process needs to be undertaken. The phenomenon of virally encoded molecules manipulating biological functions through modification can be seen elsewhere as well: examples include other HSV-1 IE genes encoding proteins, like ICP4 and ICP22, though the mechanisms of these proteins used to modify host proteins remain to be determined. However, current data seem to suggest that HSV-1 has a network of many proteins associated with protein modification of cellular host factors and functions.

TRANSCRIPTIONAL REGULATORY FUNCTION

Studies of cellular molecular biology indicate that during the evolution of eukaryotic biology, cells tend to contain complex interactions involving many molecular modification enzymes (18). One example of this is the essential mechanism of transcriptional regulation of genomic chromatin-remodeling/assembly that involves formation of histone structure, phosphorylation, ADP-ribosylation, unbiquitylation, sumoylzation, methylation and acetylation (25). As one of the pathways of HSV-1 to cell interaction, viral proteins have been shown to interact with the cellular chromatin-remodeling/assembly, which brings about the question of whether HSV-1 uses cellular gene chromatin-remodeling/assembly mechanisms to generate a unique system of gene transcriptional regulation. Of course, despite the inability of the currently available data to provide a satisfactory answer to this question, current findings that many HSV-1 transcriptional factors interact with cellular components involved in molecular modification and chromatin reorganization suggest this issue is important and worthy of further investigation.

HSV-1 DNA is presumably released into the nucleus where it subsequently persists in a circular episomal form during infection (19). This form is associated with the histone protein H3 as supported by the observation of circular genomic structures in neuronal cells carrying latent HSV-1 infections (6). Meanwhile, the transfection of HSV-1 genes into cells might result in chromatin recruitment and accumulation (14). However, different findings are reported in different experiments with regard to this event. Using a nuclease digestion assay, one group proposed to examine whether a HSV-1 DNA gene that can cause lytic infection is enwrapped by necleosomes in cells

since nucleosomes are usually not associated with viral genes (1). A chromatin immuoprecipitation (ChIP) assay has revealed that viral genes are associated with histones during lytic infection (9). Histone analysis shows methylation of lysine 4 of histone H3 (H3K4me) or acetylation of lysine 9 and 14 of histone H3 (2). Clearly, the exact structural model of HSV-1 gene chromatin formation by association with chromatin histones, instead of nucleosome formation, is not yet defined. However, viral proteins, with structures imitating cellular chromatin to some extent, are hypothesized to effect their transcriptional regulation via chromatin-remodeling/assembly. If this could be demonstrated, the observation that many HSV-1 IE proteins are capable of interacting with the cellular protein molecular modification system and that this affection on molecular modification would be very significant with regard to both viral and cellular molecular biology.

VP16 and ICP0, as viral molecules with capacities to activate HSV-1 gene transcription, might directly affect the activation of viral genes by recruiting cellular HAT molecules, and consequently lead to the development of a hypothetical system: HSV-1 gene chromatin formation → viral protein molecular modification → viral protein modification or changes in other molecular modifications → viral gene transcriptional regulation by chromatin-remodeling/assembly. There are several studies indicating the relevance of such a model. First, analysis of the HSV-1 gene shows a number of characterized elements containing CTCCC sites in IE, E and L gene promoter boundaries (2), which might be recognized by the CCCTC-binding factor (CTCF) associated with cellular chromatin transcriptional regulation. Second, ICP0 and ICP4 are reported to inhibit, at least partly, the activity of upstream or downstream histone acetyltransferases/ histone deacetylase (HAT/HDAC) (16), suggesting a cellular molecular modification mechanism associated with viral IE proteins. Finally, analysis of the HSV-1 lytic LAT gene has revealed a remarkable influence on LAT transcriptional expression resulting from alterations in chromatin structures at its 5' exon (13), further demonstrating the regulation of viral gene transcription through alterations in chromatin formation via molecular modification. In summary, we propose a potential putative viral gene transcriptional regulatory system created by the interaction of viral associated proteins with a cellular molecular modification system in cells harboring HSV-1 lytic or latent infections. Further investigation of this model could provide a better understanding of the mechanisms of HSV-1 viral gene regulation.

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