VIROLOGICA SINICA, February 2010, 25 (1):1-7
DOI 10.1007/s12250-010-3080-x

The Herpes Simplex Virus Type 1 Infected Cell Protein 22*

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Abstract: As one of the immediate-early (IE) proteins of herpes simplex virus type 1 (HSV-1), ICP22 is a multifunctional viral regulator that localizes in the nucleus of infected cells. It is required in experimental animal systems and some nonhuman cell lines, but not in Vero or HEp-2 cells. ICP22 is extensively phosphorylated by viral and cellular kinases and nucleotidylylated by casein kinase II. It has been shown to be required for efficient expression of early (E) genes and a subset of late (L) genes. ICP22, in conjunction with the UL13 kinase, mediates the phosphorylation of RNA polymerase II. Both ICP22 and UL13 are required for the activation of cdc2, the degradation of cyclins A and B and the acquisition of a new cdc2 partner, the UL42 DNA polymerase processivity factor. The cdc2–UL42 complex mediates postranscriptional modification of topoisomerase IIα in an ICP22-dependent manner to promote L gene expression. In addition, ICP22 interacts with cdk9 in a Us3 kinase dependent fashion to phosphorylate RNA polymerase II.

Key words: Herpes Simplex Virus type 1(HSV-1); ICP22; UL13

Herpes simplex virus type 1 (HSV-1) is commonly associated with infections of the mucocutaneous membranes of the mouth and eyes, of the brain, and of internal organs of infected neonates[26]. During productive infection, the 152-kb double-stranded HSV-1 genome is rapidly translocated to the nucleus where the ~80 viral genes are transcribed by the host cell RNA polymerase II (Pol II) in a temporally orchestrated program that is regulated by viral proteins [6]. Expression of the viral genes occurs in a coordinately activated cascade that consists of the sequential expression of immediate-early (IE), early (E), and late (L) genes [18]. Transcription of these genes requires the viral tegument protein VP16 but does not require new viral protein synthesis. Translation of the IE genes results in expression of six proteins, four of which (ICP0, ICP4, ICP22, and ICP27) serve to activate and regulate the ensuing expression of the E and L genes. ICP22 is a protein of 420 amino acids with a predicted molecular weight of 46,522 dalton and it migrates with an apparent size of 68 kDa [1]. As one of the IE proteins of HSV-1, ICP22 has exhibited

Received: 2009-05-31, Accepted: 2009-07-16

* Foundation items: The Startup Fund of the Hundred Talents Program of the Chinese Academy of Science (20071010-141); National Natural Science Foundation of China (30870120); Open Research Fund Program of the State Key Laboratory of Virology of China (2007003, 2009 007); Hubei Province Natural Science Foundation of Innovation Groups Project (2008CDA013).

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many functions, for example, it regulates the expression of L genes and phosphorylation of Pol II.

MODIFICATION OF ICP22

ICP22 is an extensively posttranslationally modified protein and these modifications include phosphorylation and nucleotidylylation [5,7,20,28]. Protein phosphorylation is one of the most common and important forms of protein modification since many aspects of the regulation of cell function are known to be controlled by protein phosphorylation. Proliferation, differentiation, signal transduction, and metabolism are all regulated by the balance of activity of protein kinases and protein phosphatases upon critical target proteins. ICP22 is posttranslationally modified by two viral protein kinases encoded by the Us3 and UL13 genes (primarily by UL13 and then by Us3) and by unidentified cellular kinases. However, ICP22 regulates the accumulation of a shorter mRNA and of a truncated Us3 protein kinase[34]. Both UL13 and Us3 are serine/threonine protein kinases[5]. While the majority of phosphorylated cellular proteins are modified at serine and threonine residues, many cellular proteins involved in signal transduction are phosphorylated at tyrosine residues. As ICP22 is an important viral regulatory protein, it is conceivable that it uses tyrosine phosphorylation as a way to tap into the cellular signal transduction pathways. It has been determined that the ICP22 tyrosine193 residue is required for efficient modification of the protein during viral replication in cultured cells [30]. Also ICP22 tyrosine 116, a potential phosphorylation site, is associated with virulence [9].

SUBCELLULAR LOCALIZATION OF ICP22

Many roles of the ICP22 have been described. ICP22 functions as a general repressor for certain viral and cellular promoters[12]. ICP22 affects the expression of the viral IE genes and a subset of L genes. Also ICP22 may act as a repressor of IE gene expression early in infection. For example, through interacts with specific elements upstream of the α4 gene promoter, VP16 interacts with ICP22 mediated transcriptional repression of the viral α4 gene [12, 35]. Moreover, evidences that ICP22 localizes to the transcriptional complexes that contain the viral trans-activator protein ICP4, RNA pol II, and other factors support the idea that ICP22 may functions as a transcription factor [23].

The localization of a protein determines its function. Early in infection, ICP22 localizes to small dense nuclear bodies in the infected cells. Following onset of viral DNA synthesis, ICP22 localizes to the more diffuse transcription centers associated with L gene expression[23]. Later in infection, together with UL3, UL4 and UL20.5 proteins, ICP22 re-localizes to small dense nuclear bodies[27, 42]. Our preliminary results support their interactions and subcellular localizations (unpublished data). Therefore, during HSV-1 infection, ICP22 may exert different activities at distinct nuclear sites in a temporally regulated fashion.

It was assumed that ICP22, a protein of 68 kDa, should contain some sequences which assure efficient nuclear localization of this multifunctional viral regulatory protein during the viral replication cycle. Multiple evidences have shown the ICP22 protein accumulates by itself in small, dense nuclear bodies and contains two independent sequences which are able to mediate efficient nuclear import of heterologous proteins. ICP22 contains two independent regions

with nuclear localization signal (NLS) activity. NLS1 mapped to ICP22 amino acid position 16-31 closely resembles the classical bipartite NLS originally identified in nucleoplasmin. In contrast, NLS2 is mapped to amino acid position 118–131 of ICP22 and contains multiple critical basic residues [40].

ICP22 AND THE HOST RNA POLYMERASE II

Soon after the virus enters susceptible cells, the genome is transported to the cell nucleus, where IE genes are recognized and transcribed by the host Pol II. During the process of infection, HSV-1 effectively commandeers the cell's Pol II transcription machinery to express its remaining E and L genes at high levels and in a temporally orchestrated cascade. At the same time, HSV-1 inhibits the action of Pol II on most host cell genes [38]. Evidences have shown that the IE protein ICP4 is absolutely required for efficient E and/or L transcription and the IE proteins ICP0, ICP22, and ICP27 may also help activate transcription of E and/or L genes during productive infection.

RNA polymerase II consists of at least 10 subunits ranging in size from 10 to 240 kDa. The largest subunit, which possesses catalytic activity, contains a unique carboxy-terminal structure called the C-terminal domain (CTD). During transcription, the CTD serves as a scaffold for recruiting mRNA processing and chromatin-modifying factors to the transcribing Pol II complex, and this recruitment is largely regulated by CTD phosphorylation. As a result of this modification, the large subunit is normally found in either of two states, designated IIa and IIo. IIa is hypophosphorylated, whereas IIo is heavily phosphorylated on the CTD. Thus, Pol II also exists in two forms, designated Pol IIA (containing IIa) and Pol IIO (containing IIo). Interestingly, Pol IIA and Pol IIO are associated with distinct aspects of transcription. Pol IIA is involved in transcription initiation, being the form which is preferentially recruited into preinitiation complexes. In contrast, Pol IIO is believed to be involved in transcription elongation [25, 32]. Recent evidence indicates that the CTD may also play a role in pre-mRNA processing by recruiting pre-mRNA processing factors to nascent transcripts [11, 39].

It has been proved that HSV-1 infection alters the phosphorylation state of the large subunit of Pol II [37]. Specifically, infection results in the general depletion of the IIo and IIa forms and the appearance of abundant new species which have intermediate electrophoretic mobilities. For convenience, these are referred to as a single form, designated IIi (for intermediately migrating), and have designated the form of Pol II bearing IIi as Pol III. Phosphorylation of CTD predominantly occurs on serine-2 and serine-5 (Ser-2 or Ser-5) of the CTD repeat, although recent evidence indicates that serine-7 can also be phosphorylated[16]. Phosphorylation on Ser-5 is important for promoter clearance and recruitment of mRNA capping factors, whereas phosphorylation on Ser-2 is important for efficient elongation and recruitment of polyadenylation factors.

As mentioned above, HSV-1 infection induces dramatic changes in CTD phosphorylation. Soon after infection, forms of Pol II that are phosphorylated on Ser-2 (Ser-2P Pol II) are lost in a process that involves ICP22[17, 18, 36]. Later in infection, another pathway that may involve ICP27 also contributes to loss of Ser-2P Pol II [13, 18]. And, consistent with the loss of Ser-2 phosphorylation, Pol III is predominantly phosphorylated on Ser-5[18]. The ICP22-dependent

induction of Pol III can be distinguished from the ICP22-dependent loss of Ser- 2P Pol II, as the former but not the latter requires another viral factor, the virion-associated protein kinase UL13[24]. Thus, current evidence suggests that ICP22 may mediate two distinct effects on Pol II. We have previously suggested that these effects may play a role in the shift in transcriptional specificity that occurs in HSV-1 infected cells. Using an HSV-1 ICP22 null mutant virus, it's showed that ICP22 plays an important role in the modification of Pol II. Also it has identified UL13, a virion-localized protein kinase which has been previously implicated in the posttranslational modification of ICP22 [24] affect Pol II modification. Thus, ICP22, with the UL13 protein kinase, medicate the HSV induced modification of the large subunit of RNA polymerase II to the IIi form.

Studies designed to elucidate the interaction between ICP22 and Pol II led to the discovery that ICP22 physically interacts with cyclin-dependent kinase 9 (cdk9) and that the protein complex containing ICP22 and cdk9 phosphorylated the CTD of Pol II in a viral Us3 protein kinase-dependent fashion *in vitro* [14]. Cdk9 interacts with a variety of T-type cyclins, T1, T2a, T2b, and cyclin K [19, 33]. Cdk9 and its cyclin T partners bind together and are referred to as positive transcription elongation factor b (P-TEFb). The P-TEFb complexes function to phosphorylate the CTD of Pol II and positively regulate cellular transcription [33]. It's convinced that cdk9 plays a role in the expression of the subset of genes regulated by ICP22. And there is a great possibility that cdk9 recruits ICP22 to the Pol II complex [15].

And it's identified that a region in the C-terminal half of ICP22 (residues 240 to 340) is critical for Pol II modification and the N-terminal half of the protein (residues 1 to 239) is needed for its localization to nuclear body structures. These results demonstrate that ICP22's effects on Pol II do not require its accumulation in nuclear bodies. As ICP22 is known to enhance viral L gene expression during infection of certain cultured cells, it was found that mutations in both the N- and C-terminal halves of ICP22 result in similar defects in viral L gene expression and growth in HEL cells, despite their distinctly different effects on Pol II. Thus, it uncouple ICP22's effects on Pol II from its effects on viral L gene expression. This suggests that these two functions of ICP22 may be due to distinct activities of the protein [6].

ICP22 AND CDC2

Although HSV encodes proteins required for the synthesis of its DNA, cell cycle regulators may play a significant role in establishing a more efficient environment for viral gene expression [2]. Early studies have demonstrated that HSV requires the participation of cell cycle proteins in the course of its replication [22, 41]. For example, ICP22 interacts with a novel cell cycle-regulated protein p78 [10]. Rigid regulation of cdk activity by appropriate cyclins and other regulatory proteins plays a central role in the transition of the cell from one phase of the cell cycle to the next. cdk4 and cdk6 regulated by D-type cyclins are active during early G1, whereas cdk2 regulated by cyclins E and A is active during late G1 and S phases. The G2/M transition is regulated by cdc2 (cdk1). This kinase is present throughout the cell cycle but is active in conjunction with the newly synthesized cyclins A and B. The activity of cdc2 is tightly regulated: it requires the interaction of newly synthesized cyclins

A and B; it is inactivated by phosphorylation of thr14 and thr15 by the kinases wee-1 and myt-1 [8, 31]. The cdc2 kinase is activated by the removal of thr14 and thr15 phosphates by the cdc-25C phosp- hatase and by phosphorylation of thr161 by cyclin- activating kinase complex. Cdc-25C, a key player in the activation of cdc2, must itself be activated by phosphorylation [2].

The levels of cyclins A and B were reduced to undetectable levels between 4 and 8 h after infection of HSV-1. This decrease was not observed in cells infected with mutants lacking the UL13 or the ICP22/Us1.5 genes. The stabilization of cyclins A and B in cells lacking UL13 or ICP22/Us1.5 gene products could be due to the absence of a signal for degradation of the proteins or a decrease in the expression of the virion host shutoff protein encoded by UL41 [29]. The amount of cdc2 protein was reduced after infection and, in addition, the electrophoretic mobility of a fraction of cdc2 protein differed from that of the uninfected cell. Although total protein is reduced, the activity of cdc2 kinase was significantly higher than that present in mock-infected cells and activity increased 4 to 8 h after cyclins A and B declined below detectable levels. The increased activity of cdc2 kinase without an increase in detectable protein raised the possibility that cdc2 was specifically activated from an inactive state. The increase in cdc2 activity was mediated by both UL13 and ICP 22/Us1.5 gene products. The products of the UL13 and ICP 22/Us1.5 genes mediate the hyperphosp- horylation of the cdc-25C protein. In conclusion, the disappearance of cyclins A and B and the increase in activity of the G2/M-Phase cellular kinase cdc2 in HSV-1 infected cells require expression of the ICP 22/ Us1.5 and UL13 viral genes. Since the ICP22/Us1.5 and UL13 regulatory pathway affects the expression of many genes, the specific gene whose products mediate the phenotype observed with wild-type virus remains unknown [2].

As described above, ICP22, in conjunction with UL13, mediates the degradation of cyclins A and B [2]. In the process, cdc2 acquires a new partner, the UL42 DNA polymerase accessory factor[3], and cdc2 together with UL42 binds and posttranslationally modifies topoisomerase IIα, all in an ICP22-dependent fashion [4]. Cdc2 interacts with topoisomerase II, and, moreover, proliferating cell nuclear antigen, the cellular homolog of UL42, mediates cyclin-dependent kinase substrate phosphorylation. Topoisomerase II is of particular interest because it is one of the key enzymes required for viral DNA synthesis that is not encoded by herpes viruses' but it's required for viral DNA synthesis [4]. Topoisomerase IIα could have two functions; in viral DNA synthesis and in modification of progeny DNA to facilitate late transcription. Viral DNA is made by the rolling circle model. The product consists of head-to-tail concatemers seen late in infection as huge tangles[21]. Viral DNA synthesis requires topoisomerase II activity inasmuch as enzyme inhibitors effectively block this step in viral replication. Also topoisomerase II play a role in the transcription of late genes from the progeny DNA. The intricate manner in which the virus recruits topoisomerase IIα for post-DNA synthesis expression of viral genes suggests that topoisomerase IIα is also required for untangling concatemeric DNA progeny for optimal transcription of late genes[4].

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