

EDITORIAL

HIRA in Hepatitis B Virus Minichromosome Regulation: Another Piece of the Puzzle



With 296 million infected individuals, hepatitis B virus (HBV) remains a major public health burden worldwide.¹ Those infected with HBV are at high risk of developing cirrhosis and hepatocellular carcinoma.² HBV is a small DNA virus that belongs to the Hepadnaviridae family, which specifically targets human hepatocyte. After infection, HBV releases its relaxed circular DNA (rcDNA) genome into the nucleus, where the partially double-stranded rcDNA is converted into the covalently closed circular DNA (cccDNA). cccDNA serves as the template for transcription of viral RNAs through the cellular transcription machinery.³ The persistence of cccDNA is the major challenge of current antiviral therapies: interferon- α and nucleos(t)ide reverse transcriptase inhibitors.⁴ Both treatments are effective to some extent but cannot completely block or eliminate HBV cccDNA. The current aim of chronic hepatitis B treatment is a “functional cure,” which is defined as seroclearance of hepatitis B surface antigen, undetectable serum HBV DNA, and normal liver enzymes and histology after stopping treatment, and all of them together requires a complete blockade of HBV cccDNA.⁵ Because the viral reservoir plays a central role in HBV persistence infection, solving the puzzle of HBV cccDNA is the key for a cure.

The detailed molecular mechanisms underlying the conversion of rcDNA to cccDNA remain elusive. Evidences suggest that cellular ATR-CHK1 DNA damage response, DNA repair, and chromatinization are involved, as summarized recently.³ Presumably, this multistep process involves the release of the covalently bound viral polymerase and RNA primer from the rcDNA negative strand and positive strand, respectively; the cleavage of terminally redundant sequences from the negative strand; repair of the incomplete positive strand; and ligation of both DNA strands. In addition, on the release of rcDNA into nucleus, chromatinization of rcDNA may occur concurrently with DNA repair. Loaded with histone and nonhistone proteins, HBV cccDNA is maintained stably as a minichromosome in infected hepatocytes.⁶ Its transcription is under the control of 2 enhancers and 4 promoters, which contain binding sites for ubiquitous and liver-enriched transcription factors and nuclear receptors. In addition, epigenetic modifications of HBV cccDNA minichromosomes, such as DNA methylation and histone modifications, have been implicated in regulating the transcriptional activity of HBV cccDNA.

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Locatelli et al⁷ investigated the role of HIRA in cccDNA formation and transcriptional regulation, and demonstrated an interesting and novel mechanism that

HIRA promoted the histone H3.3 deposition to maintain cccDNA chromatin assembly and transcription activity. They analyzed the cccDNA nucleoprotein structure in human hepatocytes very early after de novo infection, and found that histone deposition on incoming HBV genome and viral transcripts expression began within hours. Knocking down of HIRA showed no effect on protein free-rcDNA, the precursor of cccDNA, but significantly reduced cccDNA amount, suggesting that HIRA was essential for the formation of cccDNA minichromosome. They then infected the cells with an HBx-deficient HBV virus, which could establish a transcriptional inactivated cccDNA,⁸ and compared the cccDNA formation with wild-type virus under HIRA knockdown condition. The results indicated that HBV protein neosynthesis was dispensable for HIRA and H3.3 recruitment to cccDNA. However, chromatin immunoprecipitation experiments revealed that the viral core protein (HBc) delivered by incoming virions was temporarily and physically associated to cccDNA bound HIRA. The observation that HIRA depletion after the establishment of cccDNA strongly decreased the expression of viral RNA uncovered a dual role for HIRA in both HBV cccDNA formation and transcriptional activation, by promoting deposition of serine 31 phosphorylated H3.3.

This study shed new light on the molecular maintenance and regulation of HBV cccDNA. Moreover, it raises interesting questions to be addressed in future studies. As a histone chaperone that deposits the histone variant H3.3 in transcriptionally active genes, it is anticipated that knockdown of HIRA could result in a global change of host gene expression. Although the authors evaluated the expression of several known HBV transcription factors, the potential influence should not be overlooked. In spite of the data showing the association of HBc to cccDNA and the close proximity of HBc with HIRA in infected cells, the role of HBc in the recruitment of HIRA is unclear. It is known that HIRA binds to incoming viral, like herpes simplex virus and cytomegalovirus, and other foreign DNAs and promotes their chromatinization via deposition of histone H3.3.⁹ Future studies using HBc-deficient virus (with HBc complementation to form the virion) or HBc-defective cccDNA mimics may reveal the mystery. In addition, it is worth noting that the amounts of HIRA recruited to wild-type and HBx-deficient cccDNA are comparable at early time points post-infection. However, knockdown of chromosome 5/6 complex to activate HBx-deficient cccDNA transcription resulted in several fold increase of HIRA association. Is there a same dramatic increase of HIRA recruitment for wild-type cccDNA when transcription is augmented? Answering those questions may reveal more

details about cccDNA transcriptional regulation. Overall, this study solved an interesting piece of the HBV cccDNA puzzle, and provides evidence for more missing pieces.

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Conflicts of interest

The authors disclose no conflicts.

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