HEPATITIS

Epigenetic control of HBV by HBx protein—releasing the break?

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Refers to Riviere, L. *et al.* HBx relieves chromatin-mediated transcriptional repression of hepatitis B viral cccDNA involving SETDB1 histone methyltransferase. *J. Hepatol.* http://dx.doi.org.10.1016/j.jhep.2015.06.023

HBV persists by depositing covalently closed circular DNA (cccDNA) into the nucleus of infected host cells. HBV X protein (HBx) is a crucial determinant for regulating HBV activity, but the molecular details have not been fully understood. A new role for HBx in regulating cccDNA transcription has now been identified.

Hepatitis B infection is a major public health problem as millions of people worldwide carry the virus and are often infected for many years without knowing. Chronic HBV infection predisposes patients to severe liver disease, including decompensated cirrhosis and hepatocellular carcinoma. HBV covalently closed circular DNA (cccDNA) has a key role in the virus life cycle; cccDNA serves as a template for transcription of the HBV pre-genomic RNA and all viral genes, and is responsible for the failure of antiviral therapies as it ensures HBV persistence. Understanding the regulation of its activity is important for the control of HBV infection and ultimately its elimination. Novel molecular techniques have opened up possibilities to investigate the organization and transcriptional activity of the cccDNA that persists as a mini-chromosome *in vivo*, and shed light on the complexity of the mechanisms controlling cccDNA function. In a new study, Riviere *et al.*[1] aimed to decipher the role of chromatin and chromatin-modifier proteins on viral transcription in wild-type and HBV X protein (HBx)-deficient HBV.

> “...cccDNA ... is responsible for the failure of antiviral therapies as it ensures HBV persistence”

HBV cccDNA is organized into a chromatin-like structure associated with histone and non-histone proteins. Like cellular genes, HBV transcription might be regulated through chromatin organization and modulation of epigenetic marks. Epigenetic regulation of gene expression involves the acetylation of histones H3 and H4 or methylation at activation marks, which typically opens the chromatin structure and allows active gene transcription.[2] Alternatively, hypoacetylation of histones or methylation at inhibitory marks increases density of packaging around the DNA and silences gene transcription.[2] HBV cccDNA has long been known to have a nucleosomal organization in infected cells[3] and is thus very likely to be susceptible to epigenetic regulation. Accordingly, acetylation and deacetylation of cccDNA-bound histones are thought to regulate viral chromatin structure and subsequent gene transcription.

Transcription of cccDNA following transfection of hepatoma cell lines with a HBV genome has been shown to correlate with the recruitment of histone acetyltransferases, such as CREB-binding protein–histone acetyltransferase p300 (CREBBP–p300 HAT) and CREBBP–p300 HAT associated factor (PCAF, also known as histone acetyltransferase KAT2B), and with hyperacetylation of histones H3 and H4.[4] However, the HBV genome used was artificial, made from recircularized plasmid DNA, and the structural difference is likely to be important. Even more pertinent is that high viraemia, in patients with chronic hepatitis, correlates with hyperacetylation of histone H3 and H4 bound to cccDNA in liver biopsy samples.[4] Hyperacetylation and thus opening of HBV cccDNA chromatin-like structure allows access for liver-specific transcription factors to act in a concerted fashion for efficient HBV transcription and subsequent replication.[5] Systematic studies on the chromatinization and function of cccDNA in infection models, in which transcription is purely driven by the natural transcription template, that is cccDNA, are scarce and urgently needed.

However, viral proteins also contribute to regulating cccDNA transcriptional activity. In the woodchuck model of HBV infection it has been shown that woodchuck HBx is needed to establish productive infection in the animal.[6] The importance of HBx in the context of an infection was demonstrated in the past few years in human hepatocyte chimeric mice[7] and in cell culture.[8] Lucifora *et al.*[8] demonstrated that the HBx protein is essential to initiate and maintain transcription from HBV cccDNA and HBx-deficient HBV only replicates when expression of HBx is supplemented. The activation of HBV transcription by HBx has been proposed to be linked to chromatin modulation, as the recruitment of HBx to reconstituted cccDNA correlates with the recruitment of HBx binding partners CREBBP–p300 HAT or PCAF and with acetylation of histone H3.[9] The resulting chromatin modification opens the chromatin structure and allows transcription of cccDNA.

In their elegant study, Riviere *et al.*[1] took advantage of novel infection systems established after the Na^+-taurocholate cotransporting polypeptide was identified as the HBV host entry receptor and compared wild-type and HBx-deficient HBV. Their experiments confirmed that in the absence of HBx, HBV cccDNA is transcriptionally

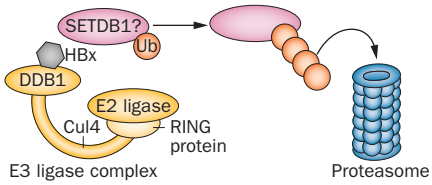

Figure 1 | HBx might direct inhibitory factors to degradation. HBx directs substrates to cullin 4 containing E3 ubiquitin ligase complex via interaction with DNA damage binding protein 1.[10] SETDB1 might be one of these substrates that become ubiqitinated and subsequently degraded by the proteasome. Abbreviations: Cul4, cullin 4; DDB1; DNA damage binding protein 1; HBx, HBV X protein, Ub, ubiquitin.

silenced with a concomitant decrease in histone H3 acetylation.[1] The investigators detected methylation of H3 at inhibitory sites, an increase of H3 dimethylation and trimethylation, and the recruitment of heterochromatin protein 1 factors that correlate with condensed chromatin, all of which explains the lack of transcription. Gene knockdown of a set of key histone methyltransferase enzymes indicated that in the absence of HBx, the histone-lysine N-methyltransferase SETDB1 was responsible for the epigenetic repression of cccDNA. Expression of HBx was able to relieve this repression and allow the establishment of active chromatin.

These results indicate that HBx is able to actively inhibit methylation of the HBV transcription template via SETDB1. The study, however, doesn't answer the question whether the histone methyltransferase is inhibited or whether the enzyme or any of its cofactors are degraded. Degradation of SETDB1 seems possible, because the association of HBx to the cell proteasome and to an E3 ubiquitinase complex has been convincingly shown.[10] HBx captures DNA damage-binding protein 1 (DDB1) through a central helical fragment, and DDB1, in concert with cullin 4, is part of the E3 ubiquitinase complex (Figure 1).[10] As such, HBx is able to redirect the ubiquitin ligase activity of this complex to certain substrates, promoting their proteasomal degradation. One might now hypothesize that SETDB1 is one of the substrates directed to proteasomal degradation; however, follow-up studies are needed to investigate this theory. The study also does not explain whether the function of HBx is to recruit transcription activating factors such as CREBBP–p300 HAT—as indicated by the earlier studies[4,9]—or to inhibit histone methylation and chromatin condensation via the methyltransferase SETDB1.[1]

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doi:10.1038/nrgastro.2015.152
Published online 1 September 2015

Competing interests
The author declares no competing interests.

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