Cellular RNA quality control functions regulate hepatitis B virus RNA steady-state levels

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I. Introduction

HBV mRNA is transcribed by host RNA pol II from cccDNA template or integrated viral genome. But little else is known about how the RNA levels are regulated, once it is transcribed. The eukaryotic cell has developed elaborate mechanisms to quality control RNA. These mechanisms mediate *degradation* of cellular coding and non-coding RNAs, that are either aberrant or in excess. It is expected that HBV RNA, would be subjected to these cellular mechanisms for its RNA metabolism, as well. Recently, Mueller et al (Hepatology, 2019) reported that cellular non-canonical polyadenylases PAPD5 and PAPD7, which are key components of the TRAMP complex for non-coding RNA process and decay, played the role in stabilization of HBV RNA. We confirmed this using tissue culture models and found that HBV RNA in cells treated with the PAPD5/7 inhibitor DHQ-1 was reduced in length initially due to 3' end shortening. The tail shortening could occur after HBV RNA 3' end processing was finished suggesting inhibition of PAPD5/7 disrupted their "tail protecting" function. PAPD5/7 seemed to stabilize HBV RNA in both nucleus and cytoplasm. They were physically associated with HBV RNA and the polyadenylating activity of PAPD5 was potently inhibited by DHQ-1 in an *in vitro* assay. Both the PAPD5 and PAPD7 have long and short isoforms. Immune fluorescent staining revealed that PAPD5 was dominantly residing in the nucleus, whereas PAPD7 was present in cytoplasm with the short isoform being enriched in the nucleus. Therefore it is possible that PAPD5 and PAPD7 are responsible for HBV RNA stabilization in the nucleus and cytoplasm, respectively.

However, PAPD5/7 was not directly responsible for removal of the HBV 3' end tail. To identify the functions responsible for this, and to identify additional RNA quality control factors involve in HBV mRNA turnover, we systematically knocked down key components of host RNA quality control/processing complexes in TRAMP, NEXT, PAXT, Ccr4-NOT and the nuclear Exosome. We found that not only PAPD5/7, but also ZFC3H1, CNOT7, Dis3 and Rrp6 might play direct or indirect roles to regulate the steady-state levels of HBV RNA.

II. Results

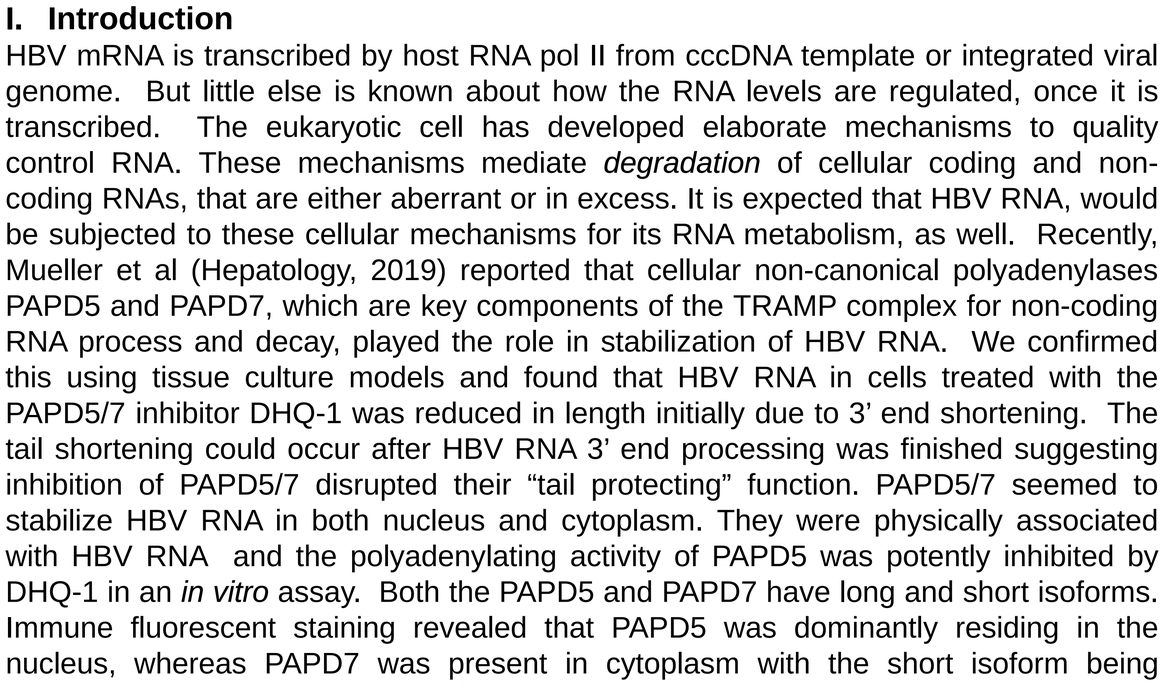

Figure 1. Multiple HBV RNAs are sensitive to PAPD5/7 inhibitor DHQ-1. HepG2-tTA25 cells were infected with Dox inducible Ad-HBV, Ad-L, Ad-S and Ad-X viruses. Three days after adeno-virus infection, Dox was added back to the culture media at time 0 to shut down viral gene transcription. DHQ-1 treatment was initiated at the same time when Dox was added. Total cellular RNA was extracted at indicated time points for Northern blot.

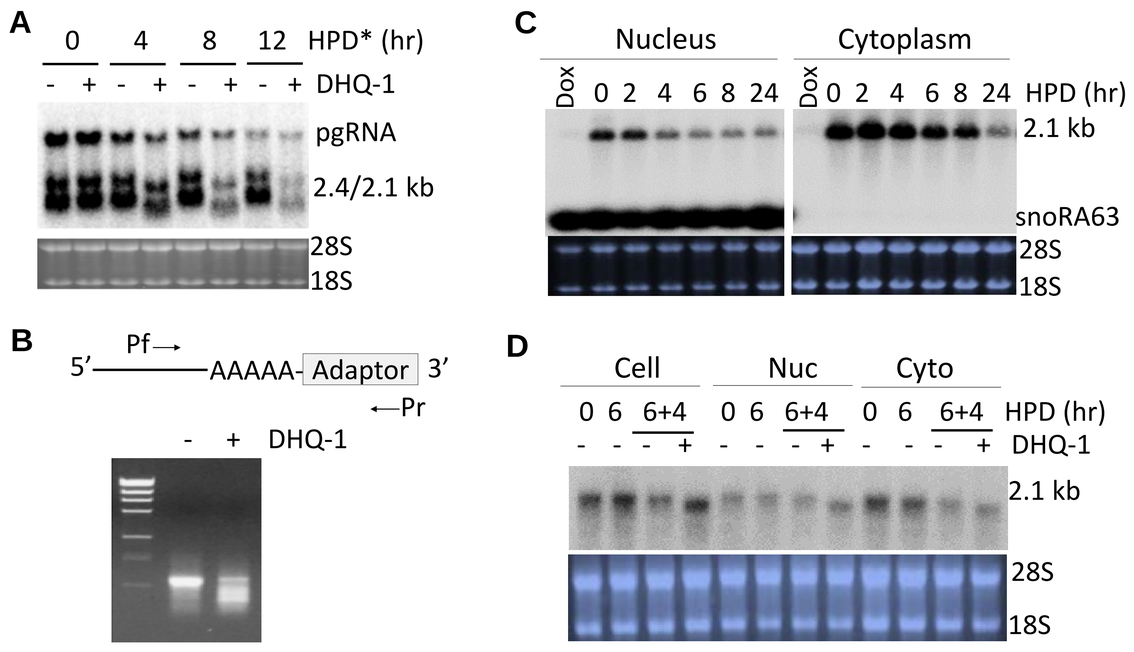

Figure 2. HBV RNA 3' end was shortened by DHQ-1 in both the nucleus and cytoplasm. (A) HBV transcription in HepAD38 cells was terminated by Dox at time 0. Treatment with DHQ-1 was started at time 0, as well. Cellular RNA was extracted for Northern. (B) RNAs derived from 8 hr in panel A were ligated to RNA adaptor and reverse transcribed for PCR using HBV specific primer and primer located in adaptor. (C) HepG2-tTA25 cells were infected with Dox inducible Ad-S. Cells were treated with Dox and fractionationed at indicated time for Northern analysis. (D) HepG2-tTA25 cells infected with tet-off Ad-S was treated with Dox for 0, 6 hr or 6 hr followed with 4 hr DHQ-1. Cells were then fractionationed for Northern blotting. HPD*: Hours Post Dox.

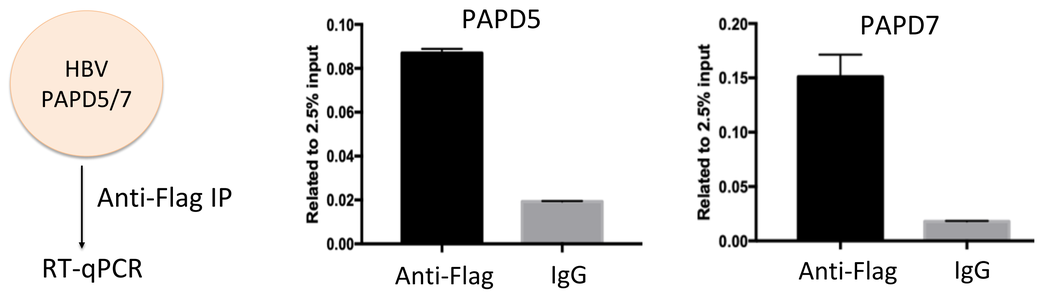

Figure 3. HBV RNA was associated with PAPD5/7. Hek293 cells were co-transfected with plasmids pCMV-S and pCMV-flagPAPD5 or pCMV-flagPAPD7. Two days after transfection, PAPD5 or PAPD7 complex was immune precipitated with M2 anti-Flag antibody or control IgG. RNA bound to beads was extracted with proteinase K and phenol:chloroform. RT-qPCR was used to quantify HBV RNA. Results were normalized based on input HBV RNA and non-relevant RNA spiked into immune precipitation.

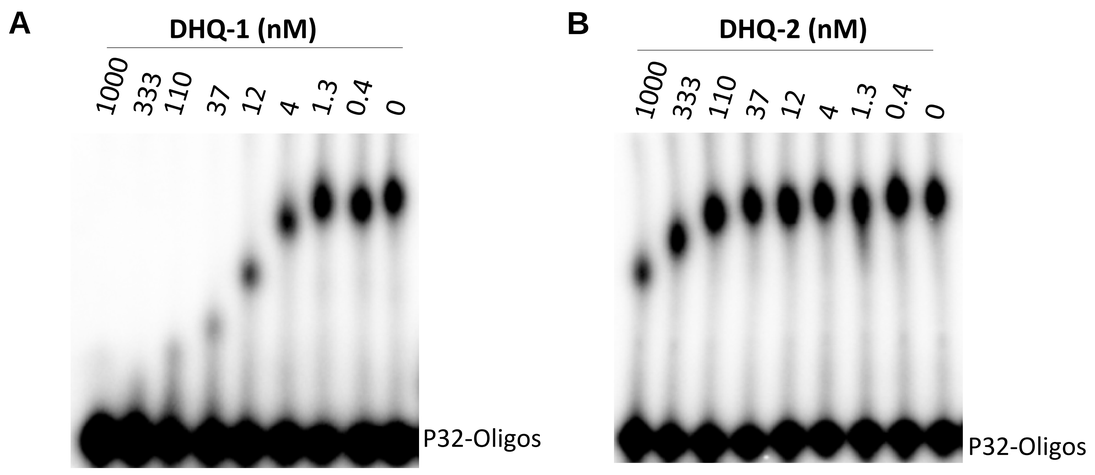

Figure 4. DHQ-1 inhibited PAPD5 polymerase activity. PAPD5 isolated from Hek293 cells was used for in vitro polyadenylation assay. In the supplement of 1 mM ATP, P32 labeled RNA oligos could be extended up to 200 nts. Application of DHQ-1 potently suppressed PAPD5 mediated polyadenylation with IC50 close to 4 nM (A). In contrast, inactive enantiomer DHQ-2 had elevated IC50 near 333 nM (B)

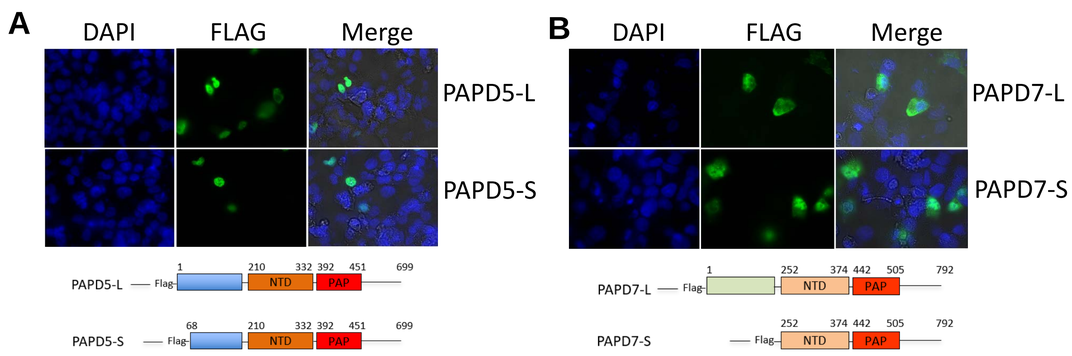

Figure 5. PAPD5 and PAPD7 may be responsible for HBV RNA stabilization in different cellular compartments. (A) HepG2 cells were transfected with pCMV-flagPAPD5-L or pCMV-flagPAPD5-S. Two days post transfection, cells were immune stained with anti-Flag antibody M2. (B) HepG2 cells transfected with long and short forms of PAPD7 were immune stained with anti-Flag, as well.

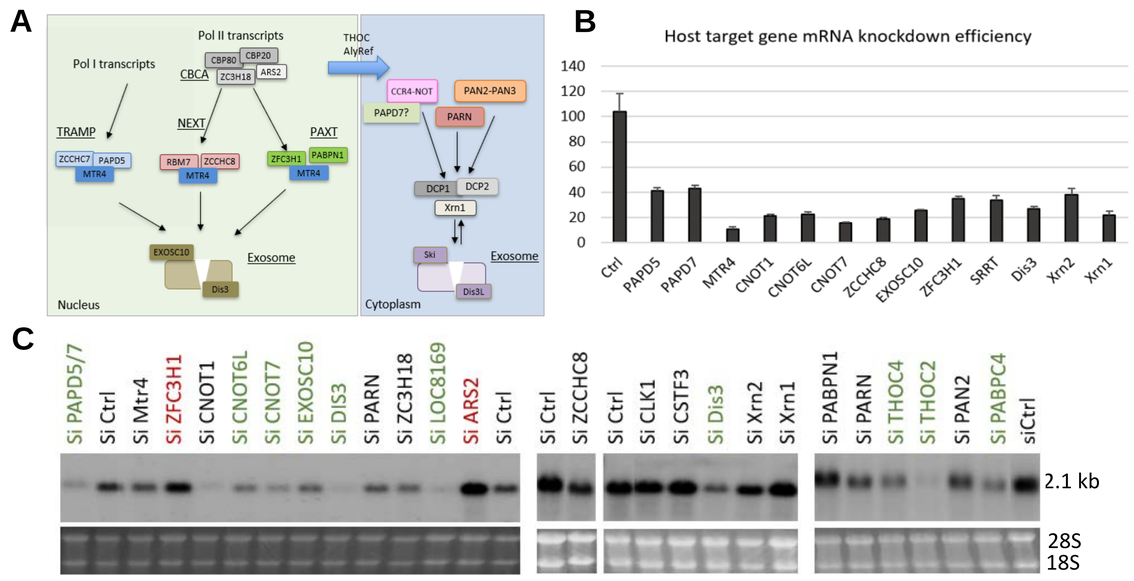

Figure 6. Other RNA quality control factors may also be involved in HBV RNA metabolism. (A) Illustration of cellular RNA quality control factors. (B) HeLa cells were transfected with siRNAs targeting host RNA processing genes. The knocking down efficacy of host genes was monitored with RT-qPCR. (C) HeLa cells having listed genes knocked down were infected with adenovirus expressing 2.1 kb HBs RNA. The effect of host factor silencing on HBs RNA expression was examined in Northern blot. Genes in green and red depict down and up regulating functions on viral RNA level, respectively.

III. Conclusions

1. Inhibition of PAPD5/7 with small molecule compound DHQ-1 accelerates degradation of multiple species of HBV RNA. However, HBx RNA seemed to be relatively insensitive to PAPD5/7 suppression.

2. DHQ-1 induced shortening of HBV RNA 3' tail takes place in both nucleus and cytoplasm. The antiviral activity of this compound might be partially dependent on its ability to block PAPD5's polyadenylating function.

3. The cellular distribution of PAPD5/7 is different suggesting they might stabilize HBV RNA at different compartments/stages.

4. In addition to PAPD5/7, components from other RNA quality control machineries such as ZFC3H1, ARS2, CNOT6/7 and Dis3 play roles to regulate HBV RNA levels, as well. The process of HBV mRNA maturation may behave more like a non coding RNA than a traditional mRNA.