Phosphorylation-Mediated interaction of HCV NS5A and the cellular YB-1 controls HCV propagation and the early stage of viral RNA replication

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About 130-150 million individuals worldwide are chronically infected by hepatitis C virus (HCV); HCV infection has become a leading cause of liver cirrhosis and hepatocellular carcinoma. The HCV life cycle relies on the cooperation of specific viral proteins and various cellular factors. The cellular protein Y-box binding protein 1 (YB-1) has recently been identified as a HCV host cofactor; however, its mechanism has not been fully characterized. The HCV nonstructural protein NS5A plays critical roles in almost every stage of HCV propagation, and has been proposed to control the switch between the defined stages of the HCV life cycle. Moreover, NS5A has recently emerged as a target for the development of novel anti-HCV drugs. Our studies have found that YB-1 not only interacts with NS5A, but also protects NS5A from degradation. Furthermore, both the NS5A/YB-1 interaction and the NS5A-stabilizing activity of YB-1 are dependent on the phosphorylation of YB-1 at serine 102 (S102). Interestingly, the YB-1 S102 site has previously been reported to be phosphorylated by Akt, which is in turn activated by HCV infection. Our study also reveals that DDX3, an YB-1-interacting partner, is another NS5A-binding protein, which plays a different role than YB-1 in HCV RNA replication and infectious virus production. Taken together, the elucidation of YB-1 participation in the HCV life cycle has led to a proposed mechanism of efficient virus propagation via coordination of the different stages of the viral life cycle through controlling stage-wise switches in the viral life cycle. Our finding also provides a novel niche for designing strategies for the development of new anti-HCV drugs by blocking specific virus-host interactions.

Keywords: hepatitis C virus; NS5A; YB-1; DDX3; phosphorylation, protein stability; infectious virus production; HCV RNA replication


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HCV is a positive-stranded RNA virus with a 9.6-kb genome. On gaining entry to the host cell, the uncoated HCV RNA genome is first translated via the internal ribosome entry site (IRES) located in 5' nontranslated region (NTR) of the viral genome. The resulting ca. 3,000-residue polyprotein is then processed by both viral and cellular proteases into three structural proteins, viz. core, E1 and E2, and seven non-structural proteins, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. The non-structural proteins NS3 to NS5B and various cellular cofactors interact to form the HCV replication complex [7]. By using the positive-strand genome as a template, the HCV replication complex synthesizes the negative-strand HCV RNA, which further act as the template for mass production of the HCV RNA genome. These processes are carried out in the virus-induced specialized membrane structure called the membranous web. The progeny HCV RNA genomes are then packaged into virions (reviewed in [8]).

NS5A is an intriguing HCV-encoded viral protein which has no known enzymatic activity [9]. NS5A is initially recognized to compose the HCV replication complex [10-13], and participates in the production of infectious virions [14-16]. More recently, NS5A is shown to be involved in the early stage of HCV RNA replication, including transient HCV RNA replication before the appearance of the membranous web [17] and the subsequent formation of the membranous web [18, 19]. NS5A is an extensively phosphorylated protein which exists in two phosphorylated forms, the hypophosphorylated (p56) and the hyperphosphorylated (p58) forms [20]. Even though the effects of phosphorylation of NS5A are not fully clarified, several studies have suggested that the ratio between the p58 and p56 forms may be important in governing switches between different stages of the HCV life cycle [9]. Moreover, NS5A has recently been demonstrated to be regulated by ubiquitin-proteasome degradation [21], a common strategy adopted by positive-stranded RNA viruses to temporally regulate the levels of specific viral proteins [22].

Although YB-1 has been demonstrated as a HCV cellular cofactor, the mechanisms of YB-1 participation and regulation in the HCV life cycle are yet to be fully clarified. To explore how YB-1 regulates the HCV life cycle, we first examined the interaction between NS5A and YB-1, and our data clearly identify YB-1 as a novel NS5A-interacting protein [23], and that YB-1 regulates HCV RNA replication. Furthermore, by comparing the effects of knockdown of YB-1, before and after HCV infection, on intracellular HCV RNA levels and the formation of the replication complexes, we presented evidences to indicate that YB-1 is involved in the early stage, but not the steady state, of HCV RNA replication. Early YB-1 involvement is further supported by the observation that YB-1 knockdown had no effects on HCV RNA levels in replicon-containing cells, a well-established model system to study steady-state HCV RNA replication. The critical role of YB-1 in HCV infectious virus production was also observed.

We noticed that the functions of YB-1 in the HCV life cycle described above parallel to those of NS5A, and that YB-1 silencing preferentially down-regulated expression of NS5A compared with the expression of other viral proteins. As YB-1 is a NS5A-interacting protein, we next investigated whether YB-1 exerts its effects on HCV life cycle through direct NS5A regulation. Data on the inhibitory effects of YB-1 silencing on the expression of ectopically expressed NS5A and the half-lives of both ectopically and replicon-expressed NS5A supported that YB-1 directly regulates NS5A via regulating the stability of the protein.

Both HCV infection and NS5A expression activate the PI3K/Akt singling pathway [24, 25], which mediates the YB-1 S102 phosphorylation. As phosphorylation of YB-1 at S102 has been reported to modulate YB-1 physiological functions [26], we then investigated whether YB-1 regulation of NS5A stability is also mediated by YB-1 S102 phosphorylation. Our results showed that YB-1 S102 phosphorylation is not only important for NS5A/YB-1 interaction, but is also crucial for the NS5A-stabilizing activity of YB-1, indicating that YB-1-mediated NS5A stabilization via S102 phosphorylation forms a feedback loop, and is signaling-regulated. In other words, our observations suggest that HCV fine-tunes the levels of NS5A through some cell signaling pathways, possibly the PI3K/Akt pathway, that mediate YB-1 S102 phosphorylation to fulfill the distinct needs at different stages of the HCV life cycle. Additionally, it is noteworthy that YB-1 not only regulates total NS5A levels but also differentially modulates the expression of the hyper- (p58) and hypophosphorylated (p56) forms of NS5A. Since the p58/p56 ratio has been proposed to control the switching between different stages of the HCV life cycle [9], YB-1 possibly participates in coordinating the stringently regulated processes of the HCV life cycle under the control of the HCV-induced signaling pathways to achieve efficient viral propagation.
Finally, comparative analysis of the functions of YB-1 and another HCV cellular cofactor, DDX3, in the different stages of the viral life cycle revealed that DDX3 and YB-1 play rather different roles in HCV propagation. The result is surprising because DDX3 and YB-1 are interacting partners in non-infected and HCV-infected cells (C. H. Chao and Y. H. Wu Lee, unpublished data and ref. [27]), and both factors associate with core, NS3, NS5A and the HCV RNA [4, 23, 27-30], implying that they function as a complex at least in some steps of the HCV life cycle. However, while DDX3 promotes HCV IRES-mediated translation [31, 32], we found that YB-1 moderately inhibits HCV translation early in the

Figure 1. Involvement of YB-1, DDX3 and viral NS5A in the regulation of the HCV life cycle. In the proposed mechanism, HCV infection first activates the PI3K/Akt pathway to phosphorylate YB-1 at serine 102. The S102-phosphorylated YB-1 interacts with and stabilizes the viral NS5A, triggering the involvement of YB-1 in each stage of HCV life cycle, as summarized in the scheme. The roles of the YB-1 interacting partner, DDX3, in the HCV life cycle are also summarized. The effects of other HCV cellular cofactors and viral proteins on HCV propagation, except NS5A, are not presented here. After entry of HCV into the host cells by receptor-mediated endocytosis (step 1), the uncoated positive-strand HCV RNA is translated (step 2), with the aid of DDX3, into a polyprotein, which is then processed into individual viral proteins (step 3). YB-1 moderately inhibits HCV translation early during infection, but YB-1 may minimally sustain HCV translation under stress conditions at later stages of HCV infection. The role of NS5A in HCV IRES-mediated translation is ambiguous. Next, the translation machinery on the positive-strand HCV RNA is replaced by the HCV replication complex with a mechanism that is not fully understood (step 4) wherein NS5A is proposed to play an important role. On the other hand, the inhibitory and facilitating effects of YB-1 on HCV translation and early HCV RNA replication, respectively, implicate involvement of YB-1 in the switching between HCV translation and RNA replication. HCV RNA replication, in turn, takes place in the membranous web (step 5). In short, both NS5A and YB-1 are involved in the early stage of HCV RNA replication while the exact role of DDX3 on the establishment of HCV RNA replication remains to be elucidated. On the other hand, both NS5A and DDX3 play critical roles while YB-1 becomes dispensable in the steady-state HCV RNA replication. The HCV positive-strand RNA produced by the replication complex is then assembled into progeny virions (step 6), a transition proposed to be controlled by the phosphorylation status of NS5A. As YB-1 regulates the ratio of p58 to p56 phosphorylated forms of NS5A, YB-1 may also play a role in the RNA replication-assembly switching. Both NS5A and YB-1 participate in the final HCV virion assembly/release (step 7) while DDX3 seems to be just a bystander on this late stage of the HCV life cycle.
HCV life cycle, and that YB-1 participates in the early stage of HCV RNA replication, a feature reminiscent of NS3-mediated regulation of switching from HCV translation to RNA replication \[^{[32]}\]. After the HCV RNA replication achieves the steady-state level, where YB-1 is no longer required or involved, we propose that DDX3 takes over from YB-1 and plays a crucial role in maintaining the steady-state HCV RNA replication. Nevertheless, DDX3 seems not involved in regulating the infectious virions production, the late stage of HCV life cycle, as YB-1 does. Thus, although sharing many common properties, we showed that DDX3 and YB-1 play rather distinctive roles in each stage of the viral life cycle for incorporation into closely connected and yet different functional complexes. How HCV switches the structure, and hence functions, of its ribonucleoprotein complexes to determine the fate of a single genomic RNA remains an intriguing question worthy of further investigation.

Taken together, our major findings are summarized in a model as illustrated in Figure 1. The model not only shows how the cellular protein, YB-1, is involved in the HCV life cycle but also how HCV regulates the levels of its viral proteins by cell signaling via host cofactors. In addition, the proposed NS5A regulatory mechanism provides a new strategy for the development of novel anti-HCV drugs by blocking specific host-virus interactions.

Conflicting interests

The authors have declared that no conflict of interests exists.

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Author contributions

W.W. conceived and designed experiment, acquired and analyzed data and drafted the manuscript. Y.W. L. conceived and supervised the project, and gave the final approval of the version to be published.

Abbreviations

HCV: hepatitis C virus; YB-1: Y-box binding protein 1; S102: serine 102; mRNPs: messenger ribonucleoprotein complexes; IRES: internal ribosome entry site; NTR: nontranslated region.

References


