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Translational enhancement of HCV RNA genotype 1b by 3′-untranslated and envelope 2 protein-coding sequences

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Abstract

HCV RNA has a unique regulatory mechanism for translation. The X region of 3′-UTR and core-coding sequence regulate HCV translation. In this study, we clarified that the entire 3′-UTR also enhances HCV translation, and the envelope-coding sequence of HCV genotype 1b increases degree of this enhancement. In the luciferase reporter assay using rabbit reticulocyte lysates, translational enhancement by 3′-UTR with core to E2 regions was 25-fold higher when compared with control RNA lacking the 3′-UTR. Presence of the entire E2 sequence was important for this enhancement. This phenomenon was not due to transcript stability, and envelope protein alone did not affect translation. E2-coding sequence of genotype 1a had no effect on translation. We observed the same results in animal cell culture systems using bicistronic RNA. Structural protein-coding sequences and 3′-UTR of HCV RNA regulate viral translation, and a target for antiviral agents may be present in these regions.

Keywords: Hepatitis C virus; Internal ribosome entry site; Translation; HCV 3′-UTR; E2

Introduction

Most eukaryotic mRNAs contain a cap structure at the 5′-end and a poly (A) tail at the 3′-end. Individually or in concert, these structures play essential roles in regulating translation (Sachs et al., 1997). Association of mRNAs with a specific RNA-binding protein and communication between both ends of the RNA sequence synergistically stimulate translation initiation (Sachs and Buratowski, 1997). In yeasts, the poly (A) tail-binding protein (Pab1p) at the 3′-end associates with eIF4G, a subunit of eIF4F, which binds to the 5′-end cap, thus stimulating translation in vitro (Tarun and Sachs, 1996; Tarun et al., 1997). In humans, interaction between the 5′- and 3′-ends similarly requires poly (A)-binding protein (PABP) at the 3′-end and PABP-interacting protein (PAIP)-1 at the 5′-end (Craig et al., 1998; Tarun and Sachs, 1995). These observations suggest that circularization of mRNAs via a cap structure, cap binding proteins (e.g., eIF4E), PABP, and a poly (A) tail is important for efficient translation (the closed-loop model) (Jacobson, 1996). In contrast, some cellular and viral RNAs are translated in cap-independent manner because of their highly structured and relatively long 5′-untranslated region (UTR) (Gan et al., 1998; Iizuka et al., 1995; Jackson et al., 1995; Macejak and Sarnow, 1991). These structured *cis* elements constitute an internal ribosome entry site (IRES) that promotes internal initiation of translation.

Hepatitis C virus (HCV), which causes a variety of liver diseases in humans (Saito et al., 1990; Tsukuma et al., 1993), undergoes translation in an IRES-dependent manner, as do picorna viruses (Fukushi et al., 1994; Honda et al., 1996; Kamoshita et al., 1997; Rijnbrand et al., 1995; Tsukiyama-Kohara et al., 1992; Wang et al., 1993). At the other end of the HCV RNA, the 3′-UTR does not contain a poly (A) tail but a poly (U/C) stretch followed by a highly conserved X region at the 3′-end (Kolykhalov et al., 1996; Tanaka et al., 1995). This X

Abbreviations: HCV, hepatitis C virus; IRES, internal ribosome entry site; PTB, polypyrimidine-tract-binding protein; UTR, untranslated region; LUC, luciferase; RRL, rabbit reticulocyte lysates; IVT, in vitro translation; CAT, chloramphenicol acetyltransferase.

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region was reported to enhance the IRES-dependent translation of HCV RNA (Ito and Lai, 1999; Ito et al., 1998). Several investigations have suggested possible functions of the poly (U/C) stretch and have identified other cellular proteins binding to the 3′-UTR of HCV RNA [e.g., La (Spangberg et al., 1999, 2001; Wood et al., 2001), HuR (Spangberg et al., 2000), hnRNP C (Chung and Kaplan, 1999; Gontarek et al., 1999; Hahm et al., 1998), and L22 (Wood et al., 2001)]. Polypyrimidine-tract-binding protein (PTB) binds both the 5′-UTR and the X region. The sequence and structure of stem-loop (SL) 2 and SL3, which are binding domains in the X region, are critical for enhancement of HCV translation (Ali and Siddiqui, 1995; Ito and Lai, 1997; Ito et al., 1998). These results suggest that protein–RNA interaction between cellular proteins and the X sequence regulates HCV translation by a mechanism similar to the closed-loop model.

Much remains to be determined regarding the functions of these interactions; one group reported down-regulation of HCV RNA translation by the entire 3′-UTR (Murakami et al., 2001). Several groups have demonstrated that the presence or absence of the 3′-UTR sequence did not affect translation efficiency in HCV genotype 1a and 2b RNA (Fang and Moyer, 2000; Imbert et al., 2003; Kong and Sarnow, 2002). Furthermore, viral proteins and their coding sequences also are involved in HCV translation. HCV core protein interacts with viral sense RNA to suppress viral translation (Shimoike et al., 1999; Zhang et al., 2002), while a pyrimidine-rich sequence in the core region of HCV RNA suppresses translation (Ito and Lai, 1999). These results indicate that viral RNA, viral protein, and host cellular factors are involved in the regulation of HCV RNA translation.

In the present study, we showed that the entire 3′-UTR also enhanced HCV translation in a reporter assay using in vitro and

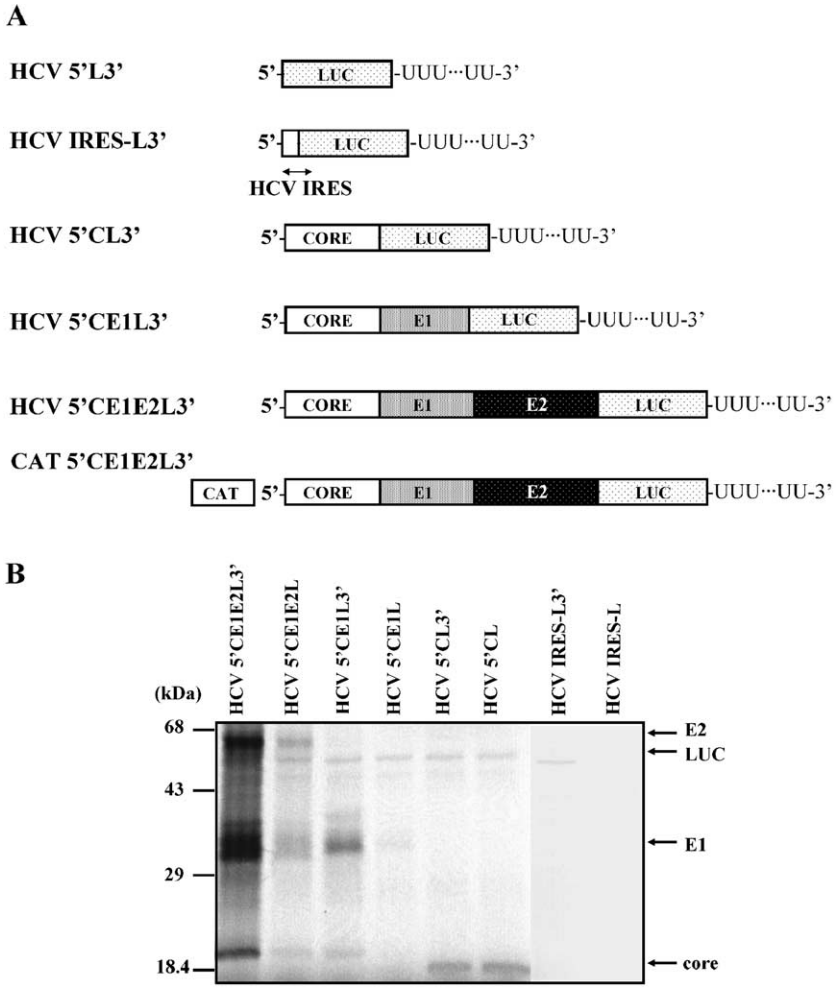

Fig. 1. Hepatitis C virus (HCV)-luciferase (LUC) chimeric RNAs and their translation products. (A) Schematic view of HCV-LUC chimeric RNAs. A T7-based plasmid (pHCV-5′L3′) containing the HCV 5′-untranslated region (UTR), core, LUC, and 3′-UTR was constructed from pCV-J4L6S. HCV core (pHCV-5′CL3′), core-to-E1 region (pHCV-5′CE1L3′), or core-to-E2 region (pHCV-5′CE1E2L3′) was inserted upstream of LUC. A plasmid containing the entire internal ribosomal entry site (IRES; nt 1 to 418), LUC, and the 3′-UTR (pHCV-IRES-L3′) was also constructed. After linearization of these plasmids by appropriate restriction enzymes, a series of HCV-LUC chimeric RNAs were synthesized by T7 RNA polymerase to generate IRES-L3′, 5′CL3′, 5′CE1L3′, and 5′CE1E2L3′, respectively. Similar RNAs lacking the 3′-UTR (IRES-L, 5′CL, 5′CE1L, and 5′CE1E2L) were also synthesized. The chloramphenicol acetyltransferase (CAT) gene was inserted between the T7 protein and the 5′-UTR of pHCV-5′CE1E2L3′ to generate CAT 5′CE1E2L3′. (B) In vitro translation (IVT) products of a series of HCV chimeric RNAs electrophoretically separated on 10% or 15% SDS-polyacrylamide gels. IVT was carried out in rabbit reticulocyte lysates (RRL) at 120 mM KCl in the presence of canine pancreatic microsomal membranes. Arrows indicate putative molecular weights of HCV core, E1, E2, and LUC proteins. Computer images were generated using Presto! Page Manager (version. 4.20.06, NewSoft Technology, Taipei, Taiwan).