Effect of NS3 and NS5B proteins on classical swine fever virus internal ribosome entry site-mediated translation and its host cellular translation

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A full-length NS3 (NS3F) and a truncated NS3 protein (NS3H) with an RNA helicase domain possess RNA helicase activity. Using an in vitro system with a monocistronic reporter RNA or DNA, containing the CSFV 5′-UTR, we observed that both NS3F and NS3H enhanced internal ribosome entry site (IRES)-mediated and cellular translation in a dose-dependent manner, but NS3 protease (NS3P) that lacks a helicase domain did not. NS3F was stronger than NS3H in promoting both translations. These results showed that viral RNA helicase could promote viral and cellular translation, and higher RNA helicase activity might be more efficient. The NS5B protein, the viral replicase, did not significantly affect the IRES-directed or cellular translation alone. NS5B significantly enhanced the stimulative effect of NS3F on both IRES-mediated and cellular translation, but did not affect that of NS3H or NS3P. This suggests that NS5B and NS3 interact via the protease domain during the enhancement of translation.

Classical swine fever virus (CSFV) is a member of the genus Pestivirus of the family Flaviviridae. Bovine viral diarrhea virus 1 (BVDV-1), BVDV-2, border disease virus (BDV) and hepatitis C virus (HCV) also belong to this family (Becher & Thiel, 2002; Cuthbert, 1994; Heinz et al., 2000). CSFV is the leading cause of classical swine fever, a highly contagious and sometimes fatal viral disease of pigs, and it can cause a considerable amount of economic loss. CSFV possesses a single plus-strand RNA genome consisting of 5′- and 3′-untranslated regions (UTRs), and a single large open reading frame (ORF) that encodes a polyprotein of approximately 3900 aa. The polyprotein (NH2-Npro-C-E1S-E2-E2S-complex-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH) is processed into mature proteins by cellular and viral proteases (Moennig & Plagemann, 1992). The 3′-UTR is most likely to be involved in the initiation of pestiviral genome replication (Isken et al., 2003; Pankraz et al., 2005; Xiao et al., 2004; Yu et al., 1999), while the 5′-UTR is able to regulate translation of the viral genomes (Fletcher & Jackson, 2002). An internal ribosome entry site (IRES) is present in the 5′-UTR, and is located between nt 40 and 350 from the 5′-terminal end of the genome. The HCV and the pestiviral IRESs direct the binding of ribosomes to the start codon even in the absence of canonical translation initiation factors (Hellen & Sarnow, 2001).

NS3 is a multifunctional protein possessing serine protease, RNA helicase and nucleoside triphosphatase (NTPase) activities, which are located in two functionally distinct domains. The N-terminal one-third of NS3 primarily serves as a protease to process the viral polyprotein. The helicase and NTPase activities are localized to the C-terminal end of the NS3 protein (Warrener & Collett, 1995; Xu et al., 1997). The HCV, BVDV and CSFV NS3 proteins have been demonstrated to have RNA helicase activity (Kim et al., 1995; Sheng et al., 2007; Tai et al., 1996; Warrener & Collett, 1995). Some plus-strand RNA viruses, such as poliovirus and rhinovirus, which lack NS3 proteins, encode a homologous protein called 2C with helicase and NTPase activity (Pfister & Wimmer, 1999). Recent evidence indicates that the NS3 protein is also important in viral replication (Gu et al., 2000; Kolykhalov et al., 2000; Piccininni et al., 2002). However, little is known about whether the CSFV NS3 protein has an effect on viral IRES-mediated and cellular translation. In this report, we investigated the effect of this protein on both IRES-mediated and cellular translations.

There have been a number of studies on IRES-mediated translation (He et al., 2003; Kallianpakou et al., 2005; Kato et al., 2002; Shimoike et al., 1999; Song et al., 2006; Zhang et al., 2002). From these studies it was found that a monocistronic reporter RNA containing viral 5′-UTR and the firefly luciferase (FLuc) is more effective than its corresponding reporter DNA in the study of IRES-directed translation (Song et al., 2006). Using standard methods, we
created a monocistronic reporter RNA. The CSFV 5′-UTR was cloned into pGEM-T vector (Promega) as described previously (Xiao et al., 2004), ligated with a PCR-generated FLuc ORF, and used as a template for T7-mediated in vitro transcription reactions as described previously (Xiao et al., 2004). Full-length NS3 (NS3F) and truncated NS3 cDNAs (NS3H) were obtained (Sheng et al., 2007) and cloned into the pcDNA-3.1 vector (Clontech) (Xiao et al., 2003). The monocistronic reporter RNA (1.0 μg) was co-transfected into PK-15 cells with the pcDNA-NS3 vector that expresses the NS3F protein. Cultivation of PK-15 cells and transfection were done as described previously (Xiao et al., 2003). As a control, an unrelated protein, β-galactosidase (LacZ) was expressed in the same vector. Cell extracts were prepared 10 h after transfection. The FLuc activity was measured with the luciferase assay kit (Promega) according to the manufacturer’s instructions. As shown in Fig. 1(a), expression of the NS3F protein increased FLuc activity compared with that observed in the LacZ expressing cell, suggesting that the NS3F protein enhanced the CSFV IRES-directed translation. It was found that the increase in FLuc activity correlated with increased levels of NS3F protein expression vector, indicating the stimulative effect of NS3F on the IRES-directed translation in a dose-dependent manner. Expression of LacZ in the same manner did not significantly affect the FLuc activity. The above results suggest that the NS3 protein might enhance IRES-directed translation. Our results are inconsistent with a previous report in which NS3 protein had no effect on IRES-directed translation (He et al., 2003). Although there is no immediate answer to explain this apparent discrepancy, it is possible that the different reporter constructs used could contribute to the discrepancies. The IRES in the reporter RNA used in our experiments is located at the 5′-terminal end of the reporter construct, in a similar location to the authentic viral IRES. In contrast, a dicistronic reporter was used in the previous report in which the IRES was inserted between the two reporter genes. It was observed that the predicted secondary structure of the IRES located at the 5′-terminal end of the reporter construct was different from that inserted between the two reporter genes (data not shown). Moreover, experiments have revealed that IRES activity can be influenced by long-range RNA–RNA interaction (Kim et al., 2003), which may have reduced the observed activity (He et al., 2003).

For further characterization of the stimulative effect of the NS3 protein on IRES-directed translation, a pcDNA-3.1 vector that expresses NS3H, a truncated NS3 helicase with the postulated helicase domain (Sheng et al., 2007) and NS5B was detected by Western blot analysis. A schematic drawing of NS3F, NS3H and NS3P is shown above. Positions of amino acids (1590, 1764 and 2272) are indicated. The pcDNA-3.1 expression vector (0.5 μg) containing the corresponding gene was used (Xiao et al., 2003). Lane ‘C’ represents the control in which the pcDNA-3.1 vector was used alone (0.5 μg). Antibodies specific to CSFV NS3 and NS5B proteins were produced by immunization of rabbits with Escherichia coli-expressed NS3 (Sheng et al., 2007) and NS5B (Xiao et al., 2006). A mouse anti-β-galactosidase antibody (Gibco-BRL) was used for the detection of β-galactosidase protein. IRES-directed translation. It was found that the increase in FLuc activity correlated with increased levels of NS3F protein expression vector, indicating the stimulative effect of NS3F on the IRES-directed translation in a dose-dependent manner. Expression of LacZ in the same manner did not significantly affect the FLuc activity.

The above results suggest that the NS3 protein might enhance IRES-directed translation. Our results are inconsistent with a previous report in which NS3 protein had no effect on IRES-directed translation (He et al., 2003). Although there is no immediate answer to explain this apparent discrepancy, it is possible that the different reporter constructs used could contribute to the discrepancies. The IRES in the reporter RNA used in our experiments is located at the 5′-terminal end of the reporter construct, in a similar location to the authentic viral IRES. In contrast, a dicistronic reporter was used in the previous report in which the IRES was inserted between two reporter genes. It was observed that the predicted secondary structure of the IRES located at the 5′-terminal end of the reporter construct was different from that inserted between the two reporter genes (data not shown). Moreover, experiments have revealed that IRES activity can be influenced by long-range RNA–RNA interaction (Kim et al., 2003), which may have reduced the observed activity (He et al., 2003).
stimulative effect of NS3H was weaker than that of NS3F (Fig. 1b). Expression of NS3F, NS3H and LacZ proteins was confirmed by immunoblot analysis using the corresponding antibody (Fig. 1c), and a dose-dependent expression of the above proteins was observed (data not shown).

CSFV NS5B protein is the viral replicase with an RNA-dependent RNA polymerase activity (Steffens et al., 1999; Xiao et al., 2006). Recent evidence indicates an interaction between the NS3 and NS5B proteins (Ishido et al., 1998; Zhang et al., 2005). To examine whether the interaction between the NS3 and NS5B proteins has an effect on the viral IRES-directed translation, we first transfected the CSFV NS5B protein expression vector into PK-15 cells (Xiao et al., 2003) together with the monocistronic reporter RNA. CSFV IRES-directed translation was not significantly affected by increasing the concentration of NS5B protein (Fig. 2a). Similar results were obtained for the CSFV NS3 protease (NS3P), which is lacking the helicase domain (Fig. 2a). The combined effect of both CSFV NS3 and NS5B on CSFV IRES-mediated translation was also examined in vivo. Transfection experiments were performed in which reporter RNA (1.0 μg) was co-transfected into PK-15 cells with 0.4 μg plasmids encoding NS3F, NS3H or NS3P, together with increasing concentrations of the NS5B protein expression vector. Results showed that the NS5B protein, in a dose-dependent manner, increased the stimulative effect of the NS3F protein on CSFV IRES-directed translation, but had no effect on translation in the presence of NS3H or NS3P (Fig. 2b). Expression of both NS5B and NS3P was confirmed by immunoblot analysis using the corresponding antibody (Fig. 1c). A dose-dependent expression of both proteins was observed (data not shown).

To test whether the above proteins affect cellular translation, a monocistronic reporter DNA was used containing the Renilla luciferase (RLuc) gene under the control of SV40 expression regulatory elements generated by PCR from pRL-SV40 vector (Promega). The reporter DNA (0.5 μg) was co-transfected into PK-15 cells with increasing concentrations of the pcDNA vectors that express NS3F, NS3H, NS5B, NS3P or LacZ alone. In comparison to the control, the NS3F and NS3H proteins had a stimulatory effect on the RLuc activity in a dose-dependent manner, while increasing the concentration of the NS5B and NS3P protein expression vectors did not significantly affect the RLuc activity (Fig. 3a and b). The NS3F protein was stronger than the NS3H protein in the enhancement of cellular translation (Fig. 3a). Expression of each of these proteins had previously been confirmed by immunoblot (Fig. 1c). These data strongly suggest that the RNA helicase, and not the RNA replicase, could enhance cellular translation. Results also showed that the NS5B protein, in a dose-dependent manner, promoted the stimulative effect of NS3F on cellular translation, but did not enhance translation in the presence of NS3H or NS3P (Fig. 3c).

To our knowledge, this is the first report on a stimulative effect of the NS3 protein on viral IRES-directed translation. However, a positive effect of RNA helicase on viral cap-directed translation has been observed previously. It was observed that RNA helicase facilitates virus gene expression and retroviral cap-directed translation (Bolinger et al., 2007; Li et al., 1999). Previous reports show that a mutant RNA helicase selectively inhibits the cap-directed translation of brome mosaic virus (Noueiry et al., 2000). Modulation of RNA structure is an essential step in many fundamental processes, including RNA synthesis, splicing, replication and translation (Kadare & Haenni, 1997). The positive effect of RNA helicase on CSFV IRES-directed translation might be beneficial to viral cytopathogenicity because of the amount that NS3 increases in cells infected with the cytopathogenic isolates (Meyers & Thiel, 1995).
NS3 RNA helicase also promotes cellular translation, consistent with previous reports in which the best characterized RNA helicase, the eukaryotic translation initiation factor eIF-4A, is believed to disrupt secondary structures in mRNA upstream of the initiation codon, thereby facilitating attachment of the 40S ribosome (Pause & Sonenberg, 1992; Rozen et al., 1990). Helicase activity is also required for efficient translation termination (Gross et al., 2007). Viral RNA helicase enhancing cellular translation has also been found in previous data (Kato et al., 2002). In fact, RNA helicases have been described as essential factors in cell development and differentiation, and some of them play a role in transcription, translation initiation and replication of viral RNA genomes (Luking et al., 1998).

Interestingly, our data show that the full-length NS3 protein is stronger than a truncated NS3 protein in promoting both virus IRES-mediated and cellular translation. Previous experiments have demonstrated that RNA helicase activity of full-length NS3 protein is higher compared with that of the truncated NS3 protein that retains the helicase domain (Sheng et al., 2007; Zhang et al., 2005). This may explain the enhanced activity of the full-length protein on translation. It is possible that higher RNA helicase activity is more efficient for translation.

This report shows that the NS5B protein is able to enhance the stimulative effect of the full-length NS3 protein on CSFV IRES-mediated translation and its host cellular translation, but that it does not affect translation in the presence of truncated NS3 proteins. This may be related to the interaction between NS3 and NS5B. It has been shown that HCV NS5B enhances RNA helicase activity of the full-length NS3 protein, but does not affect that of a truncated NS3 protein from which the protease domain has been deleted. The protease domain is required for specific NS3 and NS5B interaction, suggesting that the NS5B protein promotes RNA helicase activity of the full-length NS3 protein by binding to the NS3 protease domain (Zhang et al., 2005). Previous data have shown that NS5B forms a complex with NS3 through an amino-terminal portion of NS3 (Ishido et al., 1998). Our results suggest that the NS5B and NS3 proteins interact via the protease domain during the enhancement of translation. However, how the CSFV NS5B protein enhances the stimulative effect of the full-length NS3 protein on translation by binding to the NS3 protease domain remains unclear.

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References


