Virology 489 (2016) 51–62

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Nucleolin promotes *in vitro* translation of feline calicivirus genomic RNA

Beatriz Alvarado Hernández [a], Carlos Sandoval-Jaime [b], Stanislav V. Sosnovtsev [c], Kim Y. Green [c], Ana Lorena Gutiérrez-Escolano [a,*]

[a] Departamento de Infectómica y Patogénesis Molecular, Centro de Investigación y de Estudios Avanzados del IPN, Mexico City, Mexico
[b] Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, UNAM, Cuernavaca, Morelos, Mexico
[c] Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 17 August 2015
Returned to author for revisions
23 November 2015
Accepted 1 December 2015
Available online 18 December 2015

Keywords:
FCV
Translation
Nucleolin
AGRO100
Aptamer
RNA virus
UTRs

ABSTRACT

Feline calicivirus depends on host-cell proteins for its replication. We previously showed that knockdown of nucleolin (NCL), a phosphoprotein involved in ribosome biogenesis, resulted in the reduction of FCV protein synthesis and virus yield. Here, we found that NCL may not be involved in FCV binding and entry into cells, but it binds to both ends of the FCV genomic RNA, and stimulates its translation *in vitro*. AGRO100, an aptamer that specifically binds and inactivates NCL, caused a strong reduction in FCV protein synthesis. This effect could be reversed by the addition of full-length NCL but not by a ΔrNCL, lacking the N-terminal domain. Consistent with this, FCV infection of CrFK cells stably expressing ΔrNCL led to a reduction in virus protein translation. These results suggest that NCL is part of the FCV RNA translational complex, and that the N-terminal part of the protein is required for efficient FCV replication.

Introduction

Human caliciviruses (HuCVs), from the *Norovirus* and *Sapovirus* genera in the *Caliciviridae* family, are a major cause of epidemic gastroenteritis that affects people of all ages (Widdowson et al., 2005). Despite their impact on public health, information regarding the HuCVs replicative cycle has been limited due to the difficulty to grow them in cultured cells (Duizer et al., 2004; Jones et al., 2014; Taube et al., 2013). Feline calicivirus (FCV), a member of the genus *Vesivirus*, can be propagated efficiently in conventional cell culture (Kreutz et al., 1994), and has served as an important model for the study of calicivirus molecular biology (Papafragkou et al., 2013; Vashist et al., 2009). Similar to other positive strand RNA viruses, FCV replication involves interactions of the virus proteins with a number of host cellular factors (Li and Nagy, 2011; Liu et al., 2009). The junctional adhesion molecule 1 protein (JAM-1) is a functional receptor for FCV virions (Makino et al., 2006); some eukaryotic translation initiation factors, such as eIF4E, eIF4A, and eIF4G, promote translation of the viral RNA (Chaudhry et al., 2006; Goodfellow et al., 2005). Moreover, the polypyrimidine tract-binding protein (PTB) can bind to the 5′ terminal end of the FCV genomic and subgenomic RNAs, and is required for viral replication (Karakasiliotis et al., 2010). It was suggested that PTB functions as a negative regulator of FCV translation, promoting the synthesis of RNA (Karakasiliotis et al., 2010).

Several more studies have reported the identification of a number of host-cell proteins that interact with the 5′- and 3′- ends of calicivirus genomes (Gutierrez-Escolano et al., 2000, 2003; Vashist et al., 2012). However, the functional role of these interactions remains poorly understood. Our previous work showed that nucleolin (NCL) *in vitro* associates with the 3′ end from the FCV genomic RNA. The NCL association with the genomic RNA was further confirmed in infected cells, and a reduction of its expression by siRNA decreased protein synthesis and viral yield, confirming its role in the FCV life cycle (Cancio-Lonches et al., 2011).

NCL is a multifunctional phosphoprotein with a molecular weight of 100–110 kDa (Tajrishi et al., 2011). This protein resides primarily in the cell nucleolus; however it can also be found in the nucleus, cytoplasm and on the surface of some cells (Borer et al., 1989; Hovanessian et al., 2000; Losfeld et al., 2011; Tajrishi et al., 2011). NCL has three well-defined domains. The N-terminal

* Correspondence to: Departamento de Infectómica y Patogénesis Molecular Centro de Investigación y de Estudios Avanzados del IPN Av. IPN 2508. Col. San Pedro Zacatenco México, D.F. C.P. 07360, México. Tel.: +521 5557473800 × 5655; fax: +52 1 555747 3377.
E-mail address: alonso@cinvestav.mx (A.L. Gutiérrez-Escolano).

http://dx.doi.org/10.1016/j.virol.2015.12.001
0042-6822/

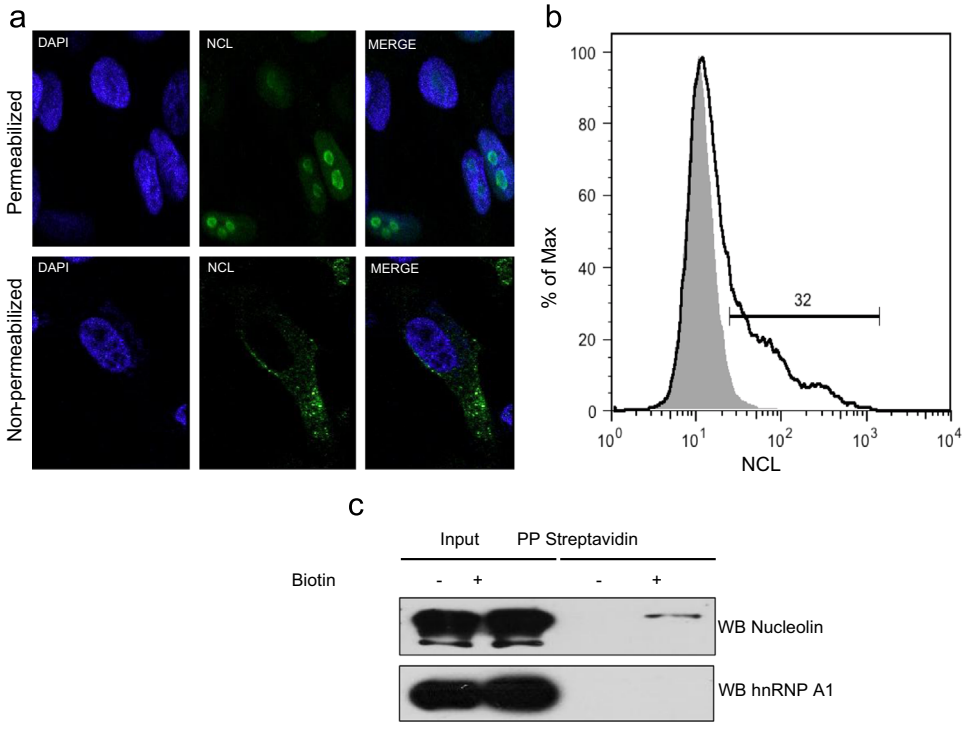

Fig. 1. Nucleolin is expressed in the surface of CrFK cells. A) Non-permeabilized and permeabilized CrFK cells with acetone, were stained with an anti-NCL antibody (green), and visualized by indirect immunofluorescence using Alexa 488 as secondary antibody. DAPI was used for nuclei staining (blue). B) Non-permeabilized CrFK cells were incubated with anti-NCL (black line) or anti-rabbit IgG antibodies (gray shaded area), used as an isotype control. C) CrFK cells were treated with sulfosuccinimidyl-6- (biotin-amide) hexanoate (+) or vehicle (−) and biotinylated proteins were precipitated with streptavidin-agarose, and separated by SDS-PAGE. NCL and hnRNP A1 proteins were detected by immunoblotting using specific anti-NCL and anti-hnRNP A1 antibodies respectively.

domain is involved in nuclear localization and responsible for binding rDNA and histone H1, and some ribosomal proteins (Erard et al., 1988; Ginisty et al., 1998). The central region contains an RNA-binding domain with four RNA recognition motifs (RRM), and is responsible for interactions with p53, beta-globin, Hsp90, and Bcl-XL mRNAs (Abdelmohsen et al., 2011; Jiang et al., 2006; Serin et al., 1996; Takagi et al., 2005; Wang et al., 2011; Zhang et al., 2008). The NCL C-terminal region contains the glycine-arginine-rich (GAR) domain that is essential for binding of ribosomal proteins and the telomerase RNA subunit hTERT (Bouvet et al., 1998; Khurts et al., 2004).

NCL controls a wide range of fundamental cellular processes such as ribosome biogenesis, proliferation and cellular cycle regulation (Cong et al., 2012; Ginisty et al., 1998; Ugrinova et al., 2007). On the cell surface, NCL serves as a receptor for ligands such as lactoferrin (Legrand et al., 2004), midkine protein (MK) (Take et al., 1994), pleiotrophin (PTN) (Said et al., 2005), and AGRO100, an oligonucleotide aptamer that has been shown to bind with high specificity and affinity to the NCL RRMs (Bates et al., 2009; Dahl et al., 1987; Mongelard and Bouvet, 2010; Reyes-Reyes et al., 2010; Soundararajan et al., 2009; Xu et al., 2001). The cell surface-expressed NCL is required for the efficient entry of human parainfluenza virus type 3 (HPV 3) into human lung epithelial A549 cells (Bose et al., 2004). NCL has been also identified as a receptor for the human respiratory syncytial virus (RSV) (Tayyari et al., 2011), and has been implicated as a low affinity receptor for the human immunodeficiency virus (HIV) (Said et al., 2002). The cytoplasmic NCL has been reported to interact with several viral proteins, such as hepatitis C virus (HCV) nonstructural protein 5B (NS5B), (Bouvet et al., 1998; Hirano et al., 2003; Kusakawa et al., 2007). Recently, it was shown that NCL could interact with dengue virus (DV) capsid protein, suggesting its role in viral morphogenesis (Balinsky et al., 2013).

In addition to protein–protein interactions, NCL has been shown to bind viral RNAs, such as the 5′ end of the HCV, poliovirus (PV), and rhinovirus (HRV) genomic RNAs (Lu et al., 2004). NCL interaction with the untranslated regions (UTRs) of the PV and HRV genomic RNAs has been found to stimulate translation of viral proteins (Izumi et al., 2001; Waggoner and Sarnow, 1998). On the other hand, NCL (Nsr1p in yeast), bound to the 3′ UTR of the tombusvirus (TBVS) RNA has been shown to inhibit its replication by interfering with recruitment of the viral RNA for replication (Jiang et al., 2010).

Due to the different processes that NCL modulates during viral replication, and because in our previous studies we demonstrated the requirement of NCL for an efficient FCV viral production (Cancio-Lonches et al., 2011), we wanted to investigate the specific function of this cellular protein during the different steps of the FCV replicative cycle. In the present report, we examined a role of NCL in binding and entry of FCV into the CrFK cells. We demonstrated that NCL specifically binds to the 5′ and 3′ end of the FCV genomic RNA, and stimulates its translation *in vitro*. We also showed that NCL mutants lacking the amino terminal domain (but containing RRMs) inhibit FCV RNA translation *in vitro*, and reduce viral protein synthesis and viral production during infection.

Results

Detection of NCL on the surface of CrFK cells

We previously reported that NCL associates with the FCV 3′ UTR and is required for an efficient viral replication cycle (Cancio-Lonches et al., 2011), since knockdown in CrFK cells by siRNAs caused a reduction in FCV production. To establish the specific role of NCL in the FCV replicative cycle, we analyzed first its possible role in virus binding and entry. It has been reported that JAM-1