Virus Research 169 (2012) 282–288

Contents lists available at SciVerse ScienceDirect

Virus Research

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

Short communication

Severe acute respiratory syndrome coronavirus accessory proteins 6 and 9b interact *in vivo*

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ARTICLE INFO

Article history:
Received 19 April 2012
Received in revised form 11 July 2012
Accepted 12 July 2012
Available online 20 July 2012

Keywords:
SARS coronavirus
Protein 6
Protein 9b
Protein–protein interactions
LC–MS

ABSTRACT

The 3′proximal one-third of the severe acute respiratory syndrome coronavirus (SARS-CoV) genome encodes the structural proteins and eight accessory proteins, including 3a, 3b, 6, 7a, 7b, 8a, 8b and 9b, varying in length from 39 to 274 aa which do not share significant homology with viral proteins of known coronaviruses. The SARS-CoV protein 6 is 63 amino acids in length and has been previously involved in virus pathogenicity and replication. To further analyze this functions, the interaction of SARS-CoV protein 6 with other viral and/or cellular factors has been analyzed during SARS-CoV infective cycle. Protein 6 immunoprecipitation from extracts of SARS-CoV infected cells and mass spectrometry analysis revealed an interaction of viral proteins 6 and 9b in biologically relevant conditions. This interaction has been reinforced by co-localization of both proteins in the cytoplasm of SARS-CoV infected cells.

Severe acute respiratory syndrome (SARS) has affected more than 8000 individuals and caused more than 800 deaths in 26 countries since the first case emerged in China in November 2002. The etiological agent of this disease was found to be a previously unknown coronavirus (SARS-CoV) (Drosten et al., 2003; Fouchier et al., 2003; Ksiazek et al., 2003; Lee et al., 2003; Peiris et al., 2003; Rota et al., 2003). In the last years SARS-CoV like viruses have been found circulating in bats from several continents (Drexler et al., 2010; Lau et al., 2005; Quan et al., 2010; Rihtaric et al., 2010) and bats have been described as putative reservoirs of SARS-CoV (Calisher et al., 2006; Li et al., 2005). Thus, the possibility of SARS reoccurrence remains.

Coronaviruses are a family of enveloped viruses with an infectious single-stranded positive-sense RNA genome of ~30 kb. The SARS-CoV genome organization is similar to that of other coronaviruses. The 5′-proximal two-thirds of the genome encode gene 1, essentially involved in viral RNA synthesis, whereas the 3′-proximal one-third of the genome encodes the structural proteins (spike, S; envelope, E; membrane, M and nucleocapsid, N) and eight accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b and 9b) varying in length from 39 to 274 aa, which do not share significant homology with viral proteins of known coronaviruses (Narayanan et al., 2008). Some of these accessory proteins, 3a, 6, 7a, 7b and 9b have been described as structural proteins (Huang et al., 2006, 2007; Ito et al., 2005; Schaecher et al., 2007; Xu et al., 2009).

Although the function of many of the accessory proteins remains unclear, SARS-CoV protein 6 is one of the best characterized accessory proteins. SARS-CoV protein 6 is 63 amino acids in length, its mRNA is present in SARS-CoV infected cells (Snijder et al., 2003) and a minimal transcription regulatory sequence is located upstream of the gene 6 open reading frame (ORF6). Evidence for the presence of protein 6 in clinical specimens has been provided (Chan et al., 2005), and antibodies against its C-terminus have been detected in SARS patients sera (Chow et al., 2006). Protein 6 has been found to localize to the rough endoplasmic reticulum (ER) and Golgi apparatus in transfected cells and in a vesicle-associated intracellular distribution in SARS-CoV infected cells (Geng et al., 2005; Gunalan et al., 2011; Kumar et al., 2007; Pewe et al., 2005). Analysis of a recombinant mouse hepatitis virus (MHV) encoding SARS-CoV protein 6 has demonstrated that it enhances virulence of an attenuated murine coronavirus (Pewe et al., 2005). Protein 6 co-immunoprecipitates with viral RNAs and accelerates replication of a mouse coronavirus (Tangudu et al., 2007). Previous data have shown the intracellular membrane localization of protein 6 in recombinant MHV infected cells (Pewe et al., 2005) and suggested its possible role in the membrane-associated events of coronavirus replication cycle (Tangudu et al., 2007), including viral RNA

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http://dx.doi.org/10.1016/j.virusres.2012.07.012

A

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1         10         20         30         40         50         60
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MFHLVDFQVTIAEILIIIMRTFRIAIWNLDVIISSIVRQLFKPLTKKNYSELDDEEPMELDYP
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B

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1         10         20         30         40         50         60         70         80         90
---------.---------.---------.---------.---------.---------.---------.---------.---------.---------
MDPNQTNVVPPALHLVDPQIQLTITRMEDAMGQGQNSADPKVYPIILRLGSQLSLSMARRNLDSLEARAFQSTPIVVQMTKLATTEELPDEFVVVTAK
```

Fig. 1. Amino acid sequence and transmembrane domains of SARS-coronavirus proteins 6 and 9b. (A) Amino acid sequence of protein 6 is shown in black. The transmembrane domain is highlighted in gray. (B) Amino acid sequence of protein 9b. The amino acids involved in the hydrophobic lipid-binding tunnel are highlighted in gray.

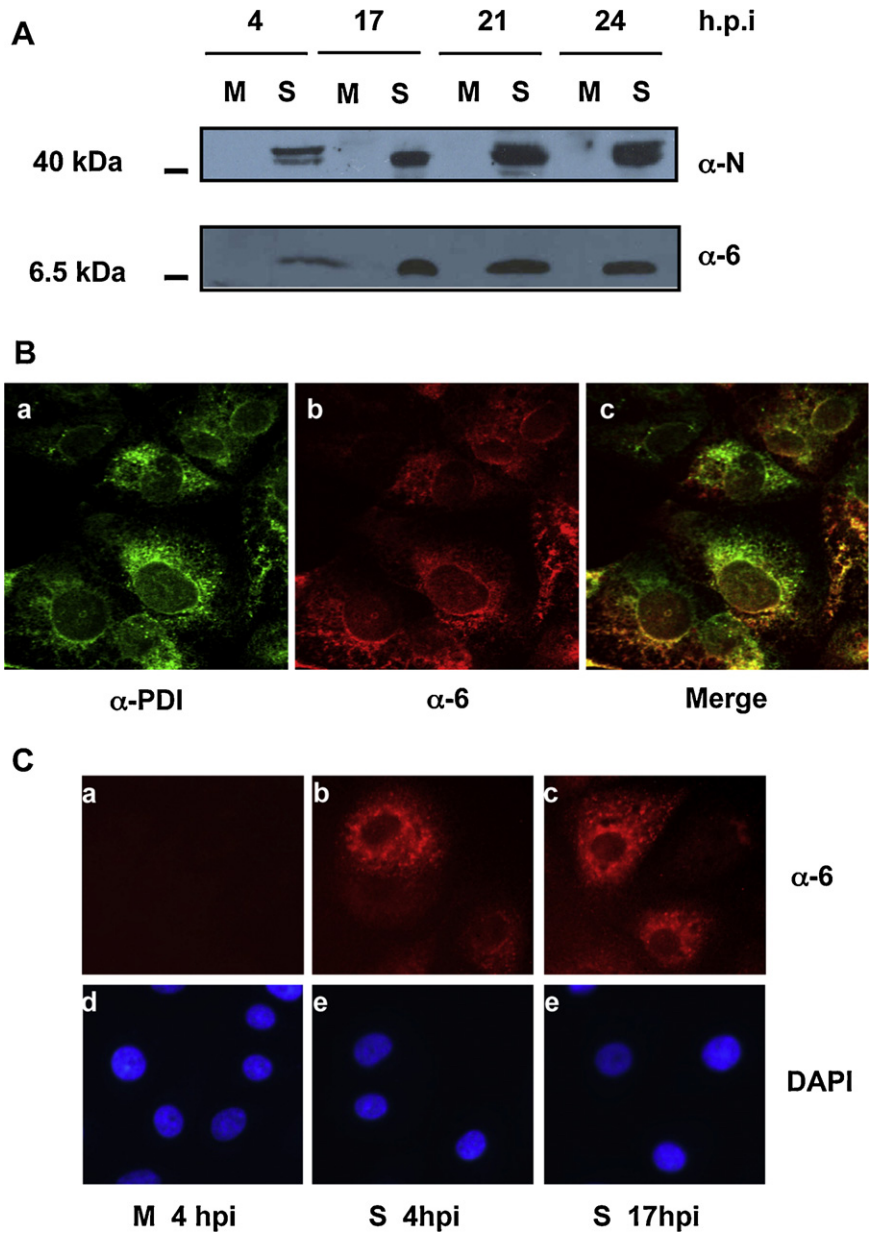

Fig. 2. SARS-CoV protein 6 expression and subcellular localization. Vero E6 cells were infected with SARS-CoV (S) or mock-infected (M). At the hours post-infection indicated in the figure (h.p.i), cells were either fixed for immunofluorescence or total cells extracts were obtained in Laemmli buffer. (A) Total cell extracts were separated by SDS-PAGE and expression of nucleoprotein (N) and protein 6 (6) was analyzed by Western blot. (B) SARS-CoV infected cell cultures were fixed at 17 h.p.i. Confocal immunofluorescence of protein 6 and ER was developed with anti-PDI rabbit antibody (ER marker) and anti-protein 6 rat antibody. Data were visualized with Alexa Fluor 488-conjugated anti-rabbit (green) and TxRed-conjugated anti-rat (red) antibodies. Co-localization of protein 6 in the ER is shown in yellow (merge). (C) Protein 6 expression was detected by immunofluorescence using an anti-protein 6 rabbit antibody and Tx-Red-conjugated anti-rabbit antibody (a–c). DAPI (blue) was used for cellular nuclei staining (d–f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)