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Virus Research 133 (2008) 113–121
Virus Research
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Review

SARS coronavirus accessory proteins

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Available online 28 November 2007

Abstract

The emergence of the severe acute respiratory syndrome coronavirus (SARS-CoV) has led to a renewed interest in studying the role of accessory proteins in regulating coronavirus infections in the natural host. A significant body of evidence has accumulated in the area of SARS-CoV and host interactions that indicate that the accessory proteins might play an important role in modulating the host response to virus infection and thereby, contribute to pathogenesis. In this review, we have compiled the current knowledge about SARS-CoV accessory proteins, obtained from studies in cell culture systems, reverse genetics and animal models, to shed some light into the possible role of these proteins in the propagation and virulence of SARS-CoV in its natural host. We conclude by providing some questions for future studies that will greatly advance our knowledge about the biological significance and contributions of the accessory proteins in the development of SARS in humans.

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Keywords: RNA viruses; SARS coronavirus; SARS-CoV; Accessory proteins

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1. Introduction

Coronaviruses possess the largest genome among any RNA viruses. The genome contains several essential genes that encode the viral proteins necessary for replication, transcription and infectious virus assembly. The essential genes comprise the open reading frames 1a and 1b (ORF1ab) that are translated to produce polyproteins that are involved in viral RNA replication and transcription. In addition to these genes, the other essential genes encode the common viral structural proteins, S, E, M and N, which are involved in infectious virus assembly (Fig. 1). Interspersed between these genes in the coronavirus genome are several other genes called "group-specific or accessory genes" and their gene products are called "accessory proteins" because many studies using reverse genetics and targeted mutagenesis analysis have shown that these genes and their products are dispensable for virus growth in vitro (Curtis et al., 2002; de Haan et al., 2002; Haijema et al., 2004; Sims et al., 2005; Wesley et al., 1991; Yount et al., 2005). Nevertheless, many of these genes are still maintained in the virus genome suggesting that they might play a very important role in the survival of the virus under the natural environment of the infected host.

The SARS-CoV genome has an unusually high number of accessory genes in the 3′-end of the genome (Fig. 1). The genome

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doi:10.1016/j.virusres.2007.10.009

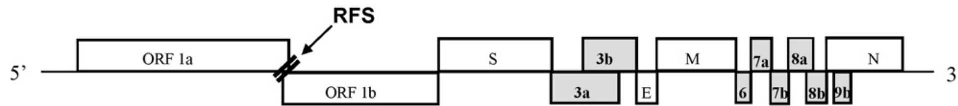

Fig. 1. Genome organization of SARS coronavirus accessory genes. The accessory genes are shown as gray boxes. The ORFs 1a and 1b comprise the SARS-CoV replicase genes. The figure is not drawn to scale. RFS: ribosomal frameshift.

contains eight accessory genes, two between S and E genes (ORFs 3a and 3b), five are located between the M and N genes (6, 7a, 7b, 8a, 8b) and one within the N gene (9b) (Fig. 1) (Marra et al., 2003; Rota et al., 2003). The sequences of most of these genes are conserved among different human and animal isolates of SARS-CoV. However, as expected, these group-specific genes have no similarity with accessory genes of coronaviruses belonging to other groups (Lai and Cavanagh, 1997). In addition, there is no significant amino-acid sequence similarity between the accessory proteins encoded by the SARS-CoV accessory genes and any known viral or cellular proteins. The field of coronavirus accessory proteins has gained considerable attention with the advent of SARS epidemic. Several studies, primarily using expression systems in cell culture, have resulted in the discovery of novel functions for the SARS-CoV accessory proteins (Table 1). The establishment of reverse genetics system for SARS-CoV has greatly facilitated the studies to determine the biological functions of these proteins in infected cells and/or animals (Almazan et al., 2006; Yount et al., 2003).

In this review, we will (1) summarize the properties, expression and subcellular localization of the individual SARS-CoV accessory proteins in cultured cells, infected cells and/or SARS patients; (2) describe studies identifying the biological function of these proteins; (3) conclude with some points about the potential implications of these discoveries for future studies.

2. SARS-CoV accessory proteins

2.1. 3a and 3b proteins

SARS-CoV 3a protein is translated from ORF 3a, located between the S and E genes, in the genome (Fig. 1). As a 274 amino-acid long protein, it is the largest of the SARS-CoV accessory proteins. Hydrophobicity analysis and topology studies have shown that 3a protein is an *O*-glycosylated protein with three transmembrane domains and has an N-terminal ectodomain and a C-terminal endodomain (Ito et al., 2005; Oostra et al., 2006; Song et al., 2005; Tan et al., 2004c; Yuan et al., 2005a). Expression studies have shown that the C-terminus of 3a protein has two intracellular protein sorting and trafficking signals, the YxxΦ and di-acidic motifs, which are important for the transport of 3a protein to the cell surface, where it subsequently undergoes endocytosis (Tan et al., 2004c). Lu et al. showed that 3a protein forms a homotetramer complex through intermolecular disulfide bridges and functions as a potassium-permeable channel-like structure (Lu et al., 2006). The biological role of the ion channel activity of 3a protein in the virus life cycle remains to be established.

Several studies have examined the properties and subcellular localization of 3a protein. In infected cells, 3a protein is localized in both intracellular and plasma membranes (Ito et al., 2005; Oostra et al., 2006; Song et al., 2005; Tan et al., 2004c; Yuan et al., 2005a). Analysis of 3a protein in infected cells and SARS patients suggested that 3a protein might be incorporated into virus particles as a result of its association with S protein (Zeng et al., 2004). Biochemical studies examining the interaction of 3a protein with other viral proteins revealed that 3a protein interacts with the structural proteins, S and M, in the Golgi apparatus proximal to the site of virus assembly and budding (Tan et al., 2004c; Yuan et al., 2005a). 3a protein also interacts with the structural protein, E, and another viral accessory protein, 7a (Tan et al., 2004c). A recent study showed that 3a protein binds to the 5′ untranslated region (UTR) of SARS genomic RNA in vitro (Sharma et al., 2007). As expected from its multiple interactions with other viral structural proteins, studies have shown clearly that 3a is a novel viral structural protein (Ito et al., 2005; Shen et al., 2005). Several studies have also demonstrated the incorporation of 3a protein into virus-like particles (VLPs), which are produced by co-expression of the virus structural proteins, S, E, M and N (Huang et al., 2006b; Shen et al., 2005). However, 3a protein is not necessary for VLP formation and SARS-CoV assembly. The production of VLPs in the absence of 3a protein expression clearly demonstrates that 3a protein is not necessary for VLP production; (Hsieh et al., 2005; Huang et al., 2006a,

Table 1
Summary of SARS-CoV accessory proteins

Accessory proteins	Incorporation into virions/VLPs	Function(s)
3a	Yes	NF-κβ ↑, JNK ↑, IL-8 ↑, RANTES ↑, ion-channel activity, apoptosis induction and cell cycle arrest.
3b	Unknown	Type I IFN production and signaling inhibition, apoptosis induction and cell cycle arrest.
ORF6	Yes	Type I IFN production and signaling inhibition.
7a	Yes	NF-κβ ↑, JNK ↑, p38 MAP kinase ↑, host translation inhibition, apoptosis induction and cell cycle arrest.
7b	Yes	No known function
8a	Unknown	No known function
8b	Unknown	No known function
9b	Unknown	No known function

(↑) up-regulation/activation.

2004; Mortola and Roy, 2004). Furthermore, the generation of a viable SARS-CoV deletion mutant lacking the 3a gene that replicates efficiently in cell culture also highlights the non-essential function of 3a protein for SARS-CoV assembly in cell culture (Yount et al., 2005).

An interesting feature of 3a protein is the finding that it is released in detergent-resistant membrane structures from SARS-CoV-infected and 3a protein-expressing cells (Huang et al., 2006b). The sorting domains, YxxΦ and di-acidic motifs, are not required for its efficient release (Huang et al., 2006b). Although the biological role of the released 3a protein in SARS-CoV life cycle remains unclear, some clues into its potential function could be obtained from observations about the highly antigenic nature of 3a protein in SARS patients. In convalescent SARS patients, antibodies against 3a protein are readily detected (Tan et al., 2004b). A strong and potentially protective humoral response is directed against the amino terminus of 3a protein in SARS patients (Zhong et al., 2006). Furthermore, immunization of rabbits with a synthetic peptide corresponding to the amino acids 15–28 from the amino terminal ectodomain of 3a protein results in the induction of neutralizing antibodies that inhibit SARS-CoV infection in VeroE6 cells (Akerstrom et al., 2006). It is worth speculating the contribution of the released 3a protein as an immune decoy in countering the host humoral response to 3a protein in SARS patients, although any such biological role of 3a protein needs experimental evidence.

Several studies have investigated the biological functions of 3a protein primarily by expression in cell culture models. In vitro expression studies in cultured cells have shown that 3a protein induces G1 phase cell cycle arrest by reducing cyclin D3 expression and inhibiting retinoblastoma protein (Rb) phosphorylation (Yuan et al., 2007) and induces apoptosis in Vero E6 cells (Law et al., 2005b). The role of cell cycle arrest and apoptosis in SARS-CoV life cycle is unclear.

The SARS-CoV-induced disease resembles the features of an acute respiratory distress (ARDS) triggered by high levels of pro-inflammatory cytokine and chemokine production. The consensus opinion, from several published studies, is that virus-induced immune pathology and the disease outcome is most probably determined by a dysregulated immune response with an intense up-regulation of pro-inflammatory cytokines and chemokines (Law et al., 2005a; Reghunathan et al., 2005; Zhang et al., 2004). The role of accessory proteins in the up-regulation of pro-inflammatory cytokines and chemokines in SARS-CoV infected cells has received some attention in some recent studies. 3a protein up-regulates the expression and secretion of fibrinogen in human lung epithelial cell line, A549 (Tan et al., 2005). This study also showed the up-regulation of fibrinogen in SARS-CoV-infected Vero E6 cells and the authors conclude that excessive production of fibrinogen might contribute to SARS pathogenesis by enhancing cytokine production (Tan et al., 2005). A recent report showed that the expression of SARS-CoV 3a protein in A549 cells activates the nuclear factor kappa B (NF-κB), a critical transcription factor involved in the activation of pro-inflammatory genes, and c-jun N-terminal kinase (JNK) (Kanzawa et al., 2006). This study also showed that 3a protein expression enhances the activation of the NF-κB-responsive chemokines, interleukin 8 (IL-8) and RANTES (CCL5), which are known to be up-regulated in SARS-CoV infection (Kanzawa et al., 2006; Law et al., 2005a).

We have similar data for the role of 3a protein in the induction of NF-κB-promoter activity in vitro. 3a protein expression in HEK 293 cells significantly augmented the activation of NF-κB and RANTES-promoter-driven luciferase reporter gene expression in the presence of double-stranded RNA (dsRNA), an intracellular intermediate of viral RNA replication (Fig. 2A and B). The enhancement of NF-κB promoter activation in the presence of 3a protein involves an intracellular sensor of dsRNA because transfection of dsRNA into cells is required for the activity of 3a protein (Fig. 3). Similarly, SARS-CoV infection in 293/ACE2 cells, stably expressing the SARS-CoV receptor, human angiotensin converting enzyme 2 (ACE2), induces the activation of NF-κB and RANTES promoters, albeit with delayed kinetics compared to the control virus, Sendai virus,

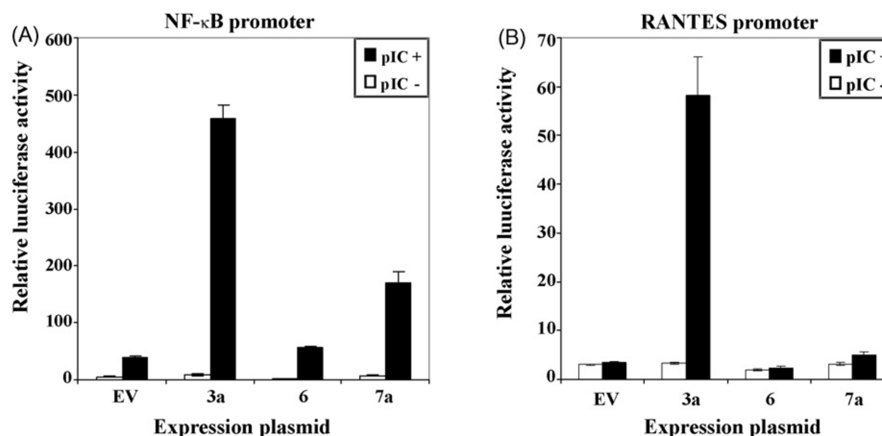

Fig. 2. 3a protein enhances dsRNA-mediated activation of NF-κB-regulated promoters. Human embryonic kidney (HEK) 293 cells were transfected with an NF-κB-driven luciferase (luc) reporter plasmid (A) or a RANTES-luc reporter plasmid (B) for the reporter assays. These cells were co-transfected with one of the indicated SCoV accessory protein expression plasmids, or a control empty vector, pcDNA 3.1 (EV) and a plasmid constitutively expressing β-galactosidase (β-gal) as an internal control. After 24 h post transfection, cells were mock-transfected, or transfected with dsRNA, poly I:C (10 μg/ml), for 6 h. Cell extracts were analyzed for luciferase activities and normalized to β-gal activity to obtain the relative luciferase activity. Triplicate samples were analyzed for each experimental group.

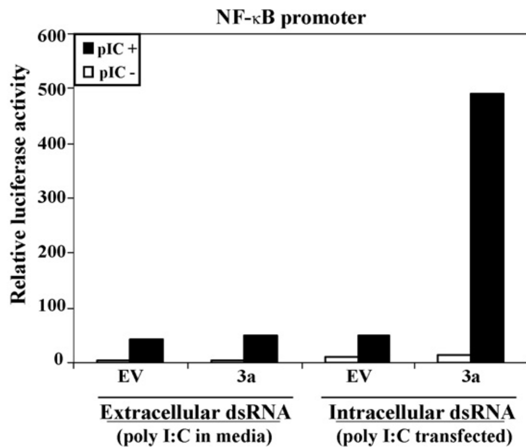

Fig. 3. 3a protein-mediated enhancement of NF-κB promoter activation requires intracellular dsRNA. HEK 293 cells were co-transfected with an NF-κB-luc reporter plasmid, β-gal plasmid along with 3a-expression plasmid or empty vector (EV). After 24 h post transfection, the cells were either mock-treated, treated with poly I:C (50 μg/ml) in the media, mock-transfected or transfected with poly I:C (10 μg/ml), for 6 h. Cell extracts were analyzed for luciferase activities and normalized to β-gal activity to obtain the relative luciferase activity. Note that 3a protein enhances the dsRNA-mediated activation of NF-κB promoter only in the dsRNA-transfected samples.

which induces a robust activation of NF-κB promoter at earlier times post-infection (Fig. 4). In contrast to our finding that showed a significant induction of NF-κB-dependent promoters in SARS-CoV-infected 293/ACE2 cells compared to mock-infected cells, other studies have reported that SARS-CoV infection does not significantly induce NF-κB-promoter-driven gene expression in 293 cells (Frieman et al., 2007a; Spiegel et al., 2005). In addition, SARS-CoV infection in Vero E6 cells also up-regulates the expression of NFKBIA, an inhibitor of the NF-κB complex (Leong et al., 2005). The discrepancy between these studies and our observation could be attributed to differences in the cell type (highly permissive 293/ACE2 cells; ∼ 80 to 90% infected) and the timing of the assay as we observed a delayed kinetics of NF-κB-dependent promoter activation compared to other studies that examined the induction at earlier times post-infection (Frieman et al., 2007a; Spiegel et al., 2005; Tang et al., 2005).

The studies described above only point to a correlation between the observations in 3a-expressing cells and SARS-CoV-infected cells. The precise contribution of 3a protein in exerting these biological functions in infected cells and its role in viral pathogenesis remains unclear and warrants further investigation using deletion mutants of SARS-CoV lacking the 3a gene.

SARS-CoV 3b protein, produced from ORF 3b, is 154-amino acids long and is reported to be localized to the nucleolus and mitochondria in expression studies (Yuan et al., 2006a, 2005c). However, a recent study using expression of GFP-tagged proteins showed that 3b protein was localized primarily in the nucleus with no evidence of mitochondrial localization (Kopecky-Bromberg et al., 2007). Immunohistochemical analysis of SARS-CoV-infected Vero E6 cells showing the presence

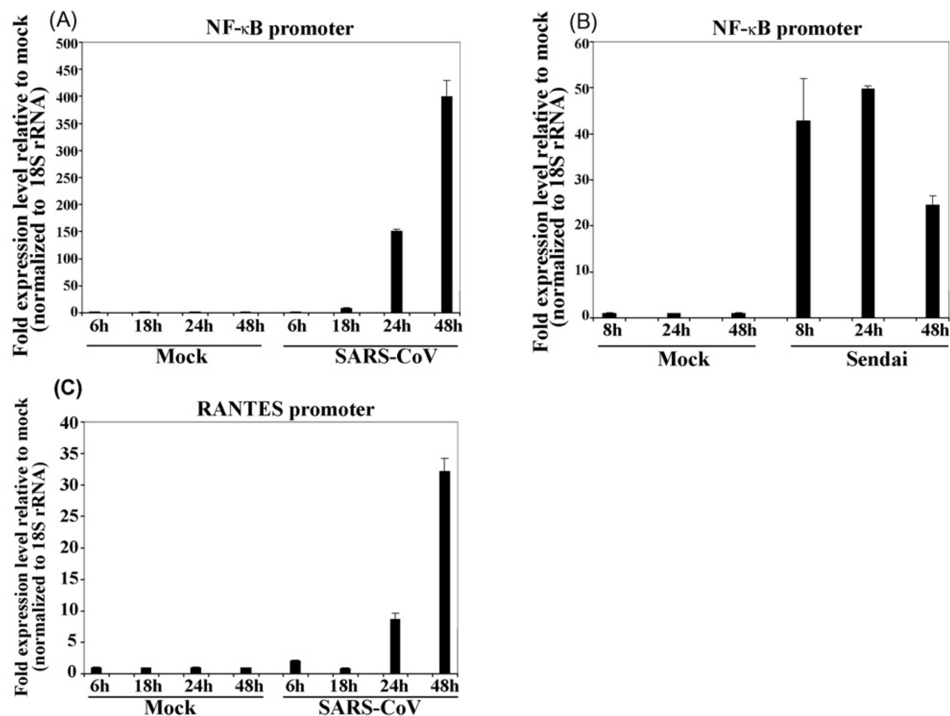

Fig. 4. SARS-CoV infection induces the activation of NF-κB-regulated promoters. 293/ACE2 cells, stably expressing the SARS-CoV receptor, human angiotensin converting enzyme 2 (ACE2), were transfected with NF-κB-luc reporter plasmid (A and B) or RANTES-luc reporter plasmid (C). After 9 h post transfection, the cells were mock-infected or infected with the Urbani strain of SARS-CoV [multiplicity of infection (MOI) = 3] or the control virus, Sendai virus (100 HA units/ml). After 18 h post-infection, RNA was extracted from the cells and luciferase mRNA levels were measured by real-time PCR analysis. The fold change in expression level, compared to mock-infected cells, is shown. The expression levels are normalized to 18S ribosomal RNA. Note the delayed kinetics in up-regulation of NF-κB and RANTES-promoter-driven luc mRNA in SARS-CoV-infected cells compared to Sendai virus infection.

of 3b protein is the only evidence for the expression of 3b protein in infected cells (Chan et al., 2005).

Expression studies showed that 3b protein induces cell growth arrest in G0/G1 phase (Yuan et al., 2005b). Another report showed that overexpression of 3b protein induces both apoptosis and necrosis in Vero E6 cells (Khan et al., 2006). 3b protein is dispensable for SARS-CoV replication in cell culture demonstrating its accessory function (Yount et al., 2005). A recent report has assigned a very important function for 3b protein in modulating the host innate immune response to SARS-CoV infection (Kopecky-Bromberg et al., 2007). This study showed that 3b protein expression inhibits both interferon production and signaling, which are key components of the antiviral innate immune response (Kopecky-Bromberg et al., 2007). The mechanism of action of 3b protein is still unclear and its interferon inhibitory function in SARS-CoV-infected cells remains to be demonstrated.

2.2. *ORF 6 protein*

SARS-CoV ORF 6 protein is a 63-amino-acid, membrane-associated protein and its expression was confirmed in virus-infected Vero E6 cells as well as in the lung and intestine tissues of SARS patients (Geng et al., 2005; Pewe et al., 2005). It is mainly localized in the endoplasmic reticulum and Golgi compartments in expressing cells (Geng et al., 2005; Pewe et al., 2005) and virus-infected cells (Frieman et al., 2007b). ORF 6 protein is incorporated into virus particles and is also released from cells expressing the protein (Huang et al., 2007). While studies have shown that ORF 6 protein is non-essential for SARS-CoV replication in cultured cells and BALB/c mice (Yount et al., 2005), several other studies have also indicated that ORF 6 protein might be involved in viral replication and inhibition of interferon (IFN) pathway (Frieman et al., 2007b; Kopecky-Bromberg et al., 2007; Pewe et al., 2005).

In a study using murine coronavirus infection model, it was shown that an attenuated mouse hepatitis coronavirus (MHV) expressing SARS-CoV ORF 6 protein replicated to a higher titer than the parental MHV in cell culture and exhibited increased virulence in mice (Pewe et al., 2005). In contrast, expression of each of the other SARS-CoV accessory proteins, 3a, 3b, 7a, 7b, and 8 using recombinant MHV did not affect virus replication and virulence, suggesting that ORF 6 protein might play a role in SARS-CoV pathogenesis. Subsequent studies using recombinant MHV also demonstrated that ORF 6 protein associated with viral RNA, co-localized with replicating viral RNA on cytoplasmic vesicles and enhanced MHV viral RNA and protein synthesis (Tangudu et al., 2007). Furthermore, studies showing the interaction of ORF 6 protein with SARS-CoV non-structural protein (nsp) 8, one of the replicase proteins encoded in ORF 1a, using a yeast two-hybrid screen and in SARS-CoV-infected cells supports the notion that ORF 6 protein could play a role in virus replication (Kumar et al., 2007). While nsp12 protein is the primary RNA-dependent RNA polymerase (RdRp) for SARS-CoV, nsp8 is a second, newly identified sequence-specific RdRp (Imbert et al., 2006). It is proposed that nsp8 protein is a low-fidelity primase producing short RNA primers utilized by the primer-dependent nsp12 for initiation of viral RNA replication. Although the biological significance of the reported interaction between ORF 6 protein and nsp8 protein is still unclear, it is reasonable to speculate that ORF 6 protein probably regulates SARS-CoV RNA replication.

A recent study revealed an important function of ORF 6 protein that showed that ORF 6 protein is a type I interferon antagonist targeting multiple steps of the IFN pathway (Frieman et al., 2007b; Kopecky-Bromberg et al., 2007). This study demonstrated that ORF 6 protein expression suppressed the IFN induction as well as the IFN signaling pathways (Kopecky-Bromberg et al., 2007).

Further studies revealed the mechanism of action of ORF 6 protein, whereby it interferes with the nuclear translocation of signal transducer and activator of transcription (STAT) 1, a critical player in the IFN signaling pathway (Frieman et al., 2007b). ORF 6 protein expression blocks the nuclear translocation of STAT1 after IFN treatment indirectly by disrupting the nuclear import complex formation through interaction of ORF 6 protein, via its C-terminus, with karyopherin alpha 2 (Frieman et al., 2007b). Karyopherin alpha 2 is a member of a complex of nuclear localization signal receptors that includes karyopherin alpha 1 and karyopherin beta 1 (Frieman et al., 2007b). The authors conclude that ORF 6 protein-mediated inhibition of the expression of STAT1-transcribed, IFN response genes, which is critical for the host to establish the antiviral state, could contribute to the immune evasion by SARS-CoV (Frieman et al., 2007b).

2.3. *7a and 7b proteins*

7a and 7b accessory proteins are produced from the bicistronic subgenomic RNA 7 of SARS-CoV (Marra et al., 2003; Rota et al., 2003). The 122-amino-acid SARS-CoV 7a protein is a type I transmembrane protein containing a 15 amino-acid signal peptide sequence, an 81 amino-acid lumenal domain, a 21 amino-acid transmembrane domain and a short C-terminal tail (Nelson et al., 2005). The crystal structure of the lumenal domain of 7a protein shows a compact seven-stranded beta sandwich, which is similar in folding and topology to members of the immunoglobulin superfamily (Nelson et al., 2005). 7a protein was shown to be present in the perinuclear region in SARS-CoV-infected cells (Fielding et al., 2006; Fielding et al., 2004; Nelson et al., 2005). There is no consensus opinion in the literature regarding the precise subcellular localization of 7a protein. While some studies have reported the co-localization of 7a protein with the endoplasmic reticulum (ER) marker, GRP94, or the intermediate compartment (ERGIC) marker Sec-31 (Fielding et al., 2006, 2004), others have shown the co-localization with the *trans*-Golgi marker, Golgin 97 (Nelson et al., 2005). Another report showed the presence of 7a protein in the Golgi compartment (Kopecky-Bromberg et al., 2006).

7a protein is incorporated into purified SARS-CoV particles (Huang et al., 2006a). Expression studies have suggested that 7a protein is incorporated into VLPs through its interaction with the viral structural proteins, M and E (Fielding et al., 2006; Huang et al., 2006a). The 7a protein also interacts with 3a protein (Tan

et al., 2004c) and S protein (Huang et al., 2006a); however, these interactions seem to be non-essential for the incorporation of 7a protein into VLPs (Huang et al., 2006a).

Several studies have reported diverse biological functions of 7a protein in cultured cells. These include induction of apoptosis in several cell lines through a caspase-dependent pathway (Tan et al., 2004a), inhibition of cellular protein synthesis, activation of p38 mitogen-activated protein kinase (Kopecky-Bromberg et al., 2006) and cell cycle arrest at the G0/G1 phase (Yuan et al., 2006b). Expressed 7a protein also interacts with small glutamine-rich tetratricopeptide repeat-containing protein with undetermined biological significance (Fielding et al., 2006). 7a protein expression also enhances pro-inflammatory cytokine production through the activation of NF-κB and JNK in A549 cells (Kanzawa et al., 2006). It is still unclear whether 7a protein exerts similar functions in virus infection.

The 7b protein, a 44-amino-acid integral membrane protein, is expressed in SARS-CoV-infected cells and is localized in the Golgi compartment (Schaecher et al., 2007). The detection of anti-7b antibody in SARS patient serum also suggests its expression in infected patients (Guo et al., 2004). 7b protein was found to be associated with intracellular virus particles and also in purified virion (Schaecher et al., 2007).

Both 7a and 7b proteins are dispensable for virus replication because SARS-CoV mutant lacking the 7a and 7b genes replicated efficiently in cell culture and mice, (Yount et al., 2005), which is also supported by the study showing a 45-nucleotide deletion in ORF 7b gene upon serial passage of SARS-CoV in Vero E6 cells (Thiel et al., 2003). Furthermore, Sims et al. showed that ORF 7a/7b can be replaced with the green fluorescent protein (GFP) generating a recombinant virus (SARS-CoV GFP) that replicated as well as wild type virus in several cell lines (Sims et al., 2005) highlighting the accessory nature of 7a and 7b proteins.

2.4. Other SARS-CoV accessory proteins

The expression of accessory proteins, 8a and 8b, produced from SARS-CoV subgenomic RNA 8, was confirmed in virus-infected cells (Keng et al., 2006). Epidemiological studies showed that the early human and animal SARS-CoV isolates contained only one intact ORF 8. During the outbreak of SARS in 2003, most human isolates of SARS-CoV had a naturally occurring 29-nt deletion in ORF 8, resulting in two discrete ORFs 8a and 8b (Guan et al., 2003). These observations suggested that the deletion of 29-nt could have some correlation with the adaptation of SARS-CoV to humans during the outbreak. However, insertion of this 29-nt sequence into human isolates using reverse genetics, which merges ORF 8a and 8b into a continuous ORF 8, had little impact on virus growth and RNA replication in cell culture (Yount et al., 2005). This data suggested the 29-nt deletion might not be responsible for the increased pathogenicity during the adaptation of SARS-CoV to humans.

8a and 8b proteins are distinct from ORF 8 protein in conformation (Keng et al., 2006). In expression studies, 8a protein interacts with S protein and 8b protein interacts with M, E, 3a and 7a proteins, while ORF 8 protein interacts with S, 3a and 7a proteins. 8b protein expression significantly down-regulated the E protein level, while the E gene mRNA level was apparently not affected (Keng et al., 2006). However, the biological function of 8a and 8b proteins is not known in the context of virus replication.

SARS-CoV 9b protein is expressed from an internal ORF in the N gene. The expression of 9b protein was demonstrated in infected cells using immunohistochemical analysis (Chan et al., 2005). The crystal structure of 9b protein revealed a novel dimeric tent-like β structure with an amphipathic surface and a central hydrophobic cavity, which binds lipid molecules that probably allows its association with intracellular vesicles. (Meier et al., 2006). The precise function of 9b protein is still not understood, although it is proposed that 9b protein might contribute to virus assembly as a membrane-attachment point for other viral proteins, like N protein (Meier et al., 2006).

3. Conclusions

The accumulation of novel information about the diverse biological functions of SARS-CoV accessory proteins has raised some interesting ideas and questions about their role in SARS-CoV pathogenesis. As most of the studies are performed using protein expressions in cell culture systems, it is imperative to validate these biological functions in the context of virus replication and pathogenesis in relevant animal models. As a first step in this direction, Yount et al. examined the role of SARS-CoV accessory proteins in virus replication in mice (Yount et al., 2005). Yount et al. showed that infection of six-week-old female BALB/c mice with the Urbani strain-derived infectious clone of SARS-CoV or its accessory gene deletion mutants resulted in no difference in virus titers in the lung at 2 days post-infection (Yount et al., 2005). However, it must be noted that this is not an ideal animal model for SARS disease because intranasal inoculation of SARS-CoV into mice results in SARS-CoV replication in the lung with no clinical signs and symptoms of SARS (Subbarao et al., 2004; Subbarao and Roberts, 2006). A recent study showed that infection of BALB/c mice with a mouse-adapted SARS-CoV (Urbani strain) resulted in a lethal infection that reproduces the clinical signs and pulmonary pathology observed in severe human cases of SARS (Roberts et al., 2007). Furthermore, two groups have independently developed an animal model for lethal infection of SARS-CoV using transgenic mice that express the functional SARS-CoV receptor, ACE2 (McCray et al., 2007; Tseng et al., 2007). Progress in this area was bolstered by the publication of a report by Rockx et al. that showed the development of two novel lethal SARS-CoV infection models in aged mice using recombinant SARS-CoV that express the spike (S) glycoprotein variants from an early human isolate and palm civet isolate (Rockx et al., 2007). Mice infected with these lethal S glycoprotein variants of SARS-CoV developed ARDS and the pathological symptoms were very similar to the pathology observed in acute human cases of SARS (Rockx et al., 2007). These exciting advances in the field combined with the availability of the reverse genetics system for SARS-CoV (Almazan

et al., 2006; Yount et al., 2003) provide us the tools to investigate the role of accessory proteins in the SARS-CoV life cycle and address some key questions:

1. What is the importance of virion-associated SARS-CoV accessory proteins, 3a, ORF 6, 7a and 7b, in the virus life cycle?
2. What is the role of the individual accessory proteins in determining the outcome of lethal SARS-CoV infection in animal models? Are the SARS-CoV accessory gene deletion mutants less virulent and if so, which accessory gene(s) is responsible for attenuation?
3. Are the deletion mutants of ORF 3b and ORF 6 more sensitive to IFN-induced inhibition of SARS-CoV infection in vitro? What is the mechanism of IFN signaling inhibition by 3b protein?
4. Does the IFN antagonizing activity of 3b and ORF 6 proteins play a significant role in modulating SARS-CoV replication and/or pathogenesis in animal models? Has SARS-CoV evolved with multiple IFN antagonizing proteins for its survival and propagation in different susceptible hosts?
5. What is the contribution of accessory genes in the up-regulation of pro-inflammatory cytokine and chemokine production in infected cells and animals? Are the deletion mutants of SARS-CoV ORFs 3a and 7a significantly impaired in inducing pro-inflammatory cytokine responses in infected cells and/or animals and if so, how does it alter the disease progression in infected animals?

Answers to some of these questions will bring us closer towards deciphering the role of these accessory genes in the coronavirus life cycle and provide valuable information that will elucidate the molecular mechanism of pathogenesis and propagation of SARS-CoV in nature.

Acknowledgements

The authors would like to thank Dr. Judith F. Aronson and Dr. Rongtuan Lin for kindly providing us the NF-κB-luciferase and RANTES-pGL3 luciferase reporter plasmids, respectively. The authors also acknowledge Dr. Kui Li for technical assistance. This work was supported by National Institutes of Health grant RO1 AI29984.

References

Akerstrom, S., Tan, Y.J., Mirazimi, A., 2006. Amino acids 15–28 in the ectodomain of SARS coronavirus 3a protein induces neutralizing antibodies. FEBS Lett. 580 (16), 3799–3803.

Almazan, F., Dediego, M.L., Galan, C., Escors, D., Alvarez, E., Ortego, J., Sola, I., Zuniga, S., Alonso, S., Moreno, J.L., Nogales, A., Capiscol, C., Enjuanes, L., 2006. Construction of a severe acute respiratory syndrome coronavirus infectious cDNA clone and a replicon to study coronavirus RNA synthesis. J. Virol. 80 (21), 10900–10906.

Chan, W.S., Wu, C., Chow, S.C., Cheung, T., To, K.F., Leung, W.K., Chan, P.K., Lee, K.C., Ng, H.K., Au, D.M., Lo, A.W., 2005. Coronaviral hypothetical and structural proteins were found in the intestinal surface enterocytes and pneumocytes of severe acute respiratory syndrome (SARS). Mod. Pathol. 18 (11), 1432–1439.

Curtis, K.M., Yount, B., Baric, R.S., 2002. Heterologous gene expression from transmissible gastroenteritis virus replicon particles. J. Virol. 76 (3), 1422–1434.

de Haan, C.A., Masters, P.S., Shen, X., Weiss, S., Rottier, P.J., 2002. The group-specific murine coronavirus genes are not essential, but their deletion, by reverse genetics, is attenuating in the natural host. Virology 296 (1), 177–189.

Fielding, B.C., Gunalan, V., Tan, T.H., Chou, C.F., Shen, S., Khan, S., Lim, S.G., Hong, W., Tan, Y.J., 2006. Severe acute respiratory syndrome coronavirus protein 7a interacts with hSGT. Biochem. Biophys. Res. Commun. 343 (4), 1201–1208.

Fielding, B.C., Tan, Y.J., Shuo, S., Tan, T.H., Ooi, E.E., Lim, S.G., Hong, W., Goh, P.Y., 2004. Characterization of a unique group-specific protein (U122) of the severe acute respiratory syndrome coronavirus. J. Virol. 78 (14), 7311–7318.

Frieman, M., Heise, M., Baric, R., 2007a. SARS coronavirus and innate immunity. Virus Res..

Frieman, M., Yount, B., Heise, M., Kopecky-Bromberg, S.A., Palese, P., Baric, R.S., 2007b. SARS-CoV ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rER/Golgi membrane. J. Virol..

Geng, H., Liu, Y.M., Chan, W.S., Lo, A.W., Au, D.M., Waye, M.M., Ho, Y.Y., 2005. The putative protein 6 of the severe acute respiratory syndrome-associated coronavirus: expression and functional characterization. FEBS Lett. 579 (30), 6763–6768.

Guan, Y., Zheng, B.J., He, Y.Q., Liu, X.L., Zhuang, Z.X., Cheung, C.L., Luo, S.W., Li, P.H., Zhang, L.J., Guan, Y.J., Butt, K.M., Wong, K.L., Chan, K.W., Lim, W., Shortridge, K.F., Yuen, K.Y., Peiris, J.S., Poon, L.L., 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302 (5643), 276–278.

Guo, J.P., Petric, M., Campbell, W., McGeer, P.L., 2004. SARS corona virus peptides recognized by antibodies in the sera of convalescent cases. Virology 324 (2), 251–256.

Haijema, B.J., Volders, H., Rottier, P.J., 2004. Live, attenuated coronavirus vaccines through the directed deletion of group-specific genes provide protection against feline infectious peritonitis. J. Virol. 78 (8), 3863–3871.

Hsieh, P.K., Chang, S.C., Huang, C.C., Lee, T.T., Hsiao, C.W., Kou, Y.H., Chen, I.Y., Chang, C.K., Huang, T.H., Chang, M.F., 2005. Assembly of severe acute respiratory syndrome coronavirus RNA packaging signal into virus-like particles is nucleocapsid dependent. J. Virol. 79 (22), 13848–13855.

Huang, C., Ito, N., Tseng, C.T., Makino, S., 2006a. Severe acute respiratory syndrome coronavirus 7a accessory protein is a viral structural protein. J. Virol. 80 (15), 7287–7294.

Huang, C., Narayanan, K., Ito, N., Peters, C.J., Makino, S., 2006b. Severe acute respiratory syndrome coronavirus 3a protein is released in membranous structures from 3a protein-expressing cells and infected cells. J. Virol. 80 (1), 210–217.

Huang, C., Peters, C.J., Makino, S., 2007. Severe acute respiratory syndrome coronavirus accessory protein 6 is a virion-associated protein and is released from 6 protein-expressing cells. J. Virol. 81 (10), 5423–5426.

Huang, Y., Yang, Z.Y., Kong, W.P., Nabel, G.J., 2004. Generation of synthetic severe acute respiratory syndrome coronavirus pseudoparticles: implications for assembly and vaccine production. J. Virol. 78 (22), 12557–12565.

Imbert, I., Guillemot, J.C., Bourhis, J.M., Bussetta, C., Coutard, B., Egloff, M.P., Ferron, F., Gorbalenya, A.E., Canard, B., 2006. A second, non-canonical RNA-dependent RNA polymerase in SARS coronavirus. EMBO J. 25 (20), 4933–4942.

Ito, N., Mossel, E.C., Narayanan, K., Popov, V.L., Huang, C., Inoue, T., Peters, C.J., Makino, S., 2005. Severe acute respiratory syndrome coronavirus 3a protein is a viral structural protein. J. Virol. 79 (5), 3182–3186.

Kanzawa, N., Nishigaki, K., Hayashi, T., Ishii, Y., Furukawa, S., Niiro, A., Yasui, F., Kohara, M., Morita, K., Matsushima, K., Le, M.Q., Masuda, T., Kannagi, M., 2006. Augmentation of chemokine production by severe acute respiratory syndrome coronavirus 3a/X1 and 7a/X4 proteins through NF-kappaB activation. FEBS Lett. 580 (30), 6807–6812.

Keng, C.T., Choi, Y.W., Welkers, M.R., Chan, D.Z., Shen, S., Gee Lim, S., Hong, W., Tan, Y.J., 2006. The human severe acute respiratory syndrome coronavirus (SARS-CoV) 8b protein is distinct from its counterpart in animal SARS-CoV and down-regulates the expression of the envelope protein in infected cells. Virology 354 (1), 132–142.

Khan, S., Fielding, B.C., Tan, T.H., Chou, C.F., Shen, S., Lim, S.G., Hong, W., Tan, Y.J., 2006. Over-expression of severe acute respiratory syndrome coronavirus 3b protein induces both apoptosis and necrosis in Vero E6 cells. Virus Res. 122 (1–2), 20–27.

Kopecky-Bromberg, S.A., Martinez-Sobrido, L., Frieman, M., Baric, R.A., Palese, P., 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J. Virol. 81 (2), 548–557.

Kopecky-Bromberg, S.A., Martinez-Sobrido, L., Palese, P., 2006. 7a protein of severe acute respiratory syndrome coronavirus inhibits cellular protein synthesis and activates p38 mitogen-activated protein kinase. J. Virol. 80 (2), 785–793.

Kumar, P., Gunalan, V., Liu, B., Chow, V.T., Druce, J., Birch, C., Catton, M., Fielding, B.C., Tan, Y.J., Lal, S.K., 2007. The nonstructural protein 8 (nsp8) of the SARS coronavirus interacts with its ORF6 accessory protein. Virology.

Lai, M.M., Cavanagh, D., 1997. The molecular biology of coronaviruses. Adv. Virus Res. 48, 1–100.

Law, H.K., Cheung, C.Y., Ng, H.Y., Sia, S.F., Chan, Y.O., Luk, W., Nicholls, J.M., Peiris, J.S., Lau, Y.L., 2005a. Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. Blood 106 (7), 2366–2374.

Law, P.T., Wong, C.H., Au, T.C., Chuck, C.P., Kong, S.K., Chan, P.K., To, K.F., Lo, A.W., Chan, J.Y., Suen, Y.K., Chan, H.Y., Fung, K.P., Waye, M.M., Sung, J.J., Lo, Y.M., Tsui, S.K., 2005b. The 3a protein of severe acute respiratory syndrome-associated coronavirus induces apoptosis in Vero E6 cells. J. Gen. Virol. 86 (Pt 7), 1921–1930.

Leong, W.F., Tan, H.C., Ooi, E.E., Koh, D.R., Chow, V.T., 2005. Microarray and real-time RT-PCR analyses of differential human gene expression patterns induced by severe acute respiratory syndrome (SARS) coronavirus infection of Vero cells. Microbes Infect. 7 (2), 248–259.

Lu, W., Zheng, B.J., Xu, K., Schwarz, W., Du, L., Wong, C.K., Chen, J., Duan, S., Deubel, V., Sun, B., 2006. Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel and modulates virus release. Proc. Natl. Acad. Sci. U.S.A. 103 (33), 12540–12545.

Marra, M.A., Jones, S.J., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S., Khattra, J., Asano, J.K., Barber, S.A., Chan, S.Y., Cloutier, A., Coughlin, S.M., Freeman, D., Girn, N., Griffith, O.L., Leach, S.R., Mayo, M., McDonald, H., Montgomery, S.B., Pandoh, P.K., Petrescu, A.S., Robertson, A.G., Schein, J.E., Siddiqui, A., Smailus, D.E., Stott, J.M., Yang, G.S., Plummer, F., Andonov, A., Artsob, H., Bastien, N., Bernard, K., Booth, T.F., Bowness, D., Czub, M., Drebot, M., Fernando, L., Flick, R., Garbutt, M., Gray, M., Grolla, A., Jones, S., Feldmann, H., Meyers, A., Kabani, A., Li, Y., Normand, S., Stroher, U., Tipples, G.A., Tyler, S., Vogrig, R., Ward, D., Watson, B., Brunham, R.C., Krajden, M., Petric, M., Skowronski, D.M., Upton, C., Roper, R.L., 2003. The Genome sequence of the SARS-associated coronavirus. Science 300 (5624), 1399–1404.

McCray Jr., P.B., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L., Netland, J., Jia, H.P., Halabi, C., Sigmund, C.D., Meyerholz, D.K., Kirby, P., Look, D.C., Perlman, S., 2007. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J. Virol. 81 (2), 813–821.

Meier, C., Aricescu, A.R., Assenberg, R., Aplin, R.T., Gilbert, R.J., Grimes, J.M., Stuart, D.I., 2006. The crystal structure of ORF-9b, a lipid binding protein from the SARS coronavirus. Structure 14 (7), 1157–1165.

Mortola, E., Roy, P., 2004. Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. FEBS Lett. 576 (1–2), 174–178.

Nelson, C.A., Pekosz, A., Lee, C.A., Diamond, M.S., Fremont, D.H., 2005. Structure and intracellular targeting of the SARS-coronavirus Orf7a accessory protein. Structure (Camb.) 13 (1), 75–85.

Oostra, M., de Haan, C.A., de Groot, R.J., Rottier, P.J., 2006. Glycosylation of the severe acute respiratory syndrome coronavirus triple-spanning membrane proteins 3a and M. J. Virol. 80 (5), 2326–2336.

Pewe, L., Zhou, H., Netland, J., Tangudu, C., Olivares, H., Shi, L., Look, D., Gallagher, T., Perlman, S., 2005. A severe acute respiratory syndrome-associated coronavirus-specific protein enhances virulence of an attenuated murine coronavirus. J. Virol. 79 (17), 11335–11342.

Reghunathan, R., Jayapal, M., Hsu, L.Y., Chng, H.H., Tai, D., Leung, B.P., Melendez, A.J., 2005. Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome. BMC Immunol. 6, 2.

Roberts, A., Deming, D., Paddock, C.D., Cheng, A., Yount, B., Vogel, L., Herman, B.D., Sheahan, T., Heise, M., Genrich, G.L., Zaki, S.R., Baric, R., Subbarao, K., 2007. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 3 (1), e5.

Rockx, B., Sheahan, T., Donaldson, E., Harkema, J., Sims, A., Heise, M., Pickles, R., Cameron, M., Kelvin, D., Baric, R., 2007. Synthetic reconstruction of zoonotic and early human severe acute respiratory syndrome coronavirus isolates that produce fatal disease in aged mice. J. Virol. 81 (14), 7410–7423.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Tong, S., Tamin, A., Lowe, L., Frace, M., DeRisi, J.L., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Ksiazek, T.G., Rollin, P.E., Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Anderson, L.J., Bellini, W.J., 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300 (5624), 1394–1399.

Schaecher, S.R., Mackenzie, J.M., Pekosz, A., 2007. The ORF7b protein of severe acute respiratory syndrome coronavirus (SARS-CoV) is expressed in virus-infected cells and incorporated into SARS-CoV particles. J. Virol. 81 (2), 718–731.

Sharma, K., Surjit, M., Satija, N., Liu, B., Chow, V.T., Lal, S.K., 2007. The 3a accessory protein of SARS coronavirus specifically interacts with the 5′UTR of its genomic RNA, Using a unique 75 amino acid interaction domain. Biochemistry 46 (22), 6488–6499.

Shen, S., Lin, P.S., Chao, Y.C., Zhang, A., Yang, X., Lim, S.G., Hong, W., Tan, Y.J., 2005. The severe acute respiratory syndrome coronavirus 3a is a novel structural protein. Biochem. Biophys. Res. Commun. 330 (1), 286–292.

Sims, A.C., Baric, R.S., Yount, B., Burkett, S.E., Collins, P.L., Pickles, R.J., 2005. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J. Virol. 79 (24), 15511–15524.

Song, H.D., Tu, C.C., Zhang, G.W., Wang, S.Y., Zheng, K., Lei, L.C., Chen, Q.X., Gao, Y.W., Zhou, H.Q., Xiang, H., Zheng, H.J., Chern, S.W., Cheng, F., Pan, C.M., Xuan, H., Chen, S.J., Luo, H.M., Zhou, D.H., Liu, Y.F., He, J.F., Qin, P.Z., Li, L.H., Ren, Y.Q., Liang, W.J., Yu, Y.D., Anderson, L., Wang, M., Xu, R.H., Wu, X.W., Zheng, H.Y., Chen, J.D., Liang, G., Gao, Y., Liao, M., Fang, L., Jiang, L.Y., Li, H., Chen, F., Di, B., He, L.J., Lin, J.Y., Tong, S., Kong, X., Du, L., Hao, P., Tang, H., Bernini, A., Yu, X.J., Spiga, O., Guo, Z.M., Pan, H.Y., He, W.Z., Manuguerra, J.C., Fontanet, A., Danchin, A., Niccolai, N., Li, Y.X., Wu, C.I., Zhao, G.P., 2005. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc. Natl. Acad. Sci. U.S.A. 102 (7), 2430–2435.

Spiegel, M., Pichlmair, A., Martinez-Sobrido, L., Cros, J., Garcia-Sastre, A., Haller, O., Weber, F., 2005. Inhibition of Beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. J. Virol. 79 (4), 2079–2086.

Subbarao, K., McAuliffe, J., Vogel, L., Fahle, G., Fischer, S., Tatti, K., Packard, M., Shieh, W.J., Zaki, S., Murphy, B., 2004. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. J. Virol. 78 (7), 3572–3577.

Subbarao, K., Roberts, A., 2006. Is there an ideal animal model for SARS? Trends Microbiol. 14 (7), 299–303.

Tan, Y.J., Fielding, B.C., Goh, P.Y., Shen, S., Tan, T.H., Lim, S.G., Hong, W., 2004a. Overexpression of 7a, a protein specifically encoded by the severe acute respiratory syndrome coronavirus, induces apoptosis via a caspase-dependent pathway. J. Virol. 78 (24), 14043–14047.

Tan, Y.J., Goh, P.Y., Fielding, B.C., Shen, S., Chou, C.F., Fu, J.L., Leong, H.N., Leo, Y.S., Ooi, E.E., Ling, A.E., Lim, S.G., Hong, W., 2004b. Profiles of antibody responses against severe acute respiratory syndrome coronavirus recombinant proteins and their potential use as diagnostic markers. Clin. Diagn Lab. Immunol. 11 (2), 362–371.

Tan, Y.J., Teng, E., Shen, S., Tan, T.H., Goh, P.Y., Fielding, B.C., Ooi, E.E., Tan, H.C., Lim, S.G., Hong, W., 2004c. A novel severe acute respiratory

syndrome coronavirus protein, u274, is transported to the cell surface and undergoes endocytosis. J. Virol. 78 (13), 6723–6734.

Tan, Y.J., Tham, P.Y., Chan, D.Z., Chou, C.F., Shen, S., Fielding, B.C., Tan, T.H., Lim, S.G., Hong, W., 2005. The severe acute respiratory syndrome coronavirus 3a protein up-regulates expression of fibrinogen in lung epithelial cells. J. Virol. 79 (15), 10083–10087.

Tang, B.S., Chan, K.H., Cheng, V.C., Woo, P.C., Lau, S.K., Lam, C.C., Chan, T.L., Wu, A.K., Hung, I.F., Leung, S.Y., Yuen, K.Y., 2005. Comparative host gene transcription by microarray analysis early after infection of the Huh7 cell line by severe acute respiratory syndrome coronavirus and human coronavirus 229E. J. Virol. 79 (10), 6180–6193.

Tangudu, C., Olivares, H., Netland, J., Perlman, S., Gallagher, T., 2007. Severe acute respiratory syndrome coronavirus protein 6 accelerates murine coronavirus infections. J. Virol. 81 (3), 1220–1229.

Thiel, V., Ivanov, K.A., Putics, A., Hertzig, T., Schelle, B., Bayer, S., Weissbrich, B., Snijder, E.J., Rabenau, H., Doerr, H.W., Gorbalenya, A.E., Ziebuhr, J., 2003. Mechanisms and enzymes involved in SARS coronavirus genome expression. J. Gen. Virol. 84 (Pt 9), 2305–2315.

Tseng, C.T., Huang, C., Newman, P., Wang, N., Narayanan, K., Watts, D.M., Makino, S., Packard, M.M., Zaki, S.R., Chan, T.S., Peters, C.J., 2007. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme 2 virus receptor. J. Virol. 81 (3), 1162–1173.

Wesley, R.D., Woods, R.D., Cheung, A.K., 1991. Genetic analysis of porcine respiratory coronavirus, an attenuated variant of transmissible gastroenteritis virus. J. Virol. 65 (6), 3369–3373.

Yount, B., Curtis, K.M., Fritz, E.A., Hensley, L.E., Jahrling, P.B., Prentice, E., Denison, M.R., Geisbert, T.W., Baric, R.S., 2003. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. Proc. Natl. Acad. Sci. U.S.A. 100 (22), 12995–13000.

Yount, B., Roberts, R.S., Sims, A.C., Deming, D., Frieman, M.B., Sparks, J., Denison, M.R., Davis, N., Baric, R.S., 2005. Severe acute respiratory syndrome coronavirus group-specific open reading frames encode nonessential functions for replication in cell cultures and mice. J. Virol. 79 (23), 14909–14922.

Yuan, X., Li, J., Shan, Y., Yang, Z., Zhao, Z., Chen, B., Yao, Z., Dong, B., Wang, S., Chen, J., Cong, Y., 2005a. Subcellular localization and membrane association of SARS-CoV 3a protein. Virus Res. 109 (2), 191–202.

Yuan, X., Shan, Y., Yao, Z., Li, J., Zhao, Z., Chen, J., Cong, Y., 2006a. Mitochondrial location of severe acute respiratory syndrome coronavirus 3b protein. Mol. Cells 21 (2), 186–191.

Yuan, X., Shan, Y., Zhao, Z., Chen, J., Cong, Y., 2005b. G0/G1 arrest and apoptosis induced by SARS-CoV 3b protein in transfected cells. Virol. J. 2, 66.

Yuan, X., Wu, J., Shan, Y., Yao, Z., Dong, B., Chen, B., Zhao, Z., Wang, S., Chen, J., Cong, Y., 2006b. SARS coronavirus 7a protein blocks cell cycle progression at G0/G1 phase via the cyclin D3/pRb pathway. Virology 346 (1), 74–85.

Yuan, X., Yao, Z., Shan, Y., Chen, B., Yang, Z., Wu, J., Zhao, Z., Chen, J., Cong, Y., 2005c. Nucleolar localization of non-structural protein 3b, a protein specifically encoded by the severe acute respiratory syndrome coronavirus. Virus Res. 114 (1–2), 70–79.

Yuan, X., Yao, Z., Wu, J., Zhou, Y., Shan, Y., Dong, B., Zhao, Z., Hua, P., Chen, J., Cong, Y., 2007. G1 phase cell cycle arrest induced by SARS-CoV 3a protein via the cyclin D3/pRb pathway. Am. J. Respir. Cell Mol. Biol. 37 (1), 9–19.

Zeng, R., Yang, R.F., Shi, M.D., Jiang, M.R., Xie, Y.H., Ruan, H.Q., Jiang, X.S., Shi, L., Zhou, H., Zhang, L., Wu, X.D., Lin, Y., Ji, Y.Y., Xiong, L., Jin, Y., Dai, E.H., Wang, X.Y., Si, B.Y., Wang, J., Wang, H.X., Wang, C.E., Gan, Y.H., Li, Y.C., Cao, J.T., Zuo, J.P., Shan, S.F., Xie, E., Chen, S.H., Jiang, Z.Q., Zhang, X., Wang, Y., Pei, G., Sun, B., Wu, J.R., 2004. Characterization of the 3a protein of SARS-associated coronavirus in infected vero E6 cells and SARS patients. J. Mol. Biol. 341 (1), 271–279.

Zhang, Y., Li, J., Zhan, Y., Wu, L., Yu, X., Zhang, W., Ye, L., Xu, S., Sun, R., Wang, Y., Lou, J., 2004. Analysis of serum cytokines in patients with severe acute respiratory syndrome. Infect. Immun. 72 (8), 4410–4415.

Zhong, X., Guo, Z., Yang, H., Peng, L., Xie, Y., Wong, T.Y., Lai, S.T., Guo, Z., 2006. Amino terminus of the SARS coronavirus protein 3a elicits strong, potentially protective humoral responses in infected patients. J. Gen. Virol. 87 (Pt 2), 369–373.