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Review

Studying classical swine fever virus: Making the best of a bad virus

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ABSTRACT

Classical swine fever (CSF) is a highly contagious and often fatal disease that affects domestic pigs and wild boars. Outbreak of CSF can cause heavy economic losses to the pig industry. The strategies to prevent, control and eradicate CSF disease are based on containing the disease through a systematic prophylactic vaccination policy and a non-vaccination stamping-out policy. The quest for prevention, control and eradication of CSF has moved research forward in academia and industry, and has produced noticeable advances in understanding fundamental aspects of the virus replication mechanisms, virulence, and led to the development of new vaccines. In this review we summarize recent progress in CSFV epidemiology, molecular features of the genome and proteome, the molecular basis of virulence, and the development of anti-virus technologies.

Contents

1. Patterns of CSF epidemics and vaccine strategies

Classical swine fever (CSF), formerly called hog cholera (HC), is an economically important, highly contagious disease of pigs, which is listed by the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and must be reported to the OIE. CSF was first recognized in Tennessee, USA in 1810 (Agriculture, 2010; OIE, 2014), and then rapidly spread around the world (reviewedin Edwards et al., 2000). Although it had been successfully eradicated from Canada (1963), the United States (1978), Australia and New Zealand, it still exacts severe impacts in Asia, South America, eastern Europe and parts of the former Soviet Union (Fig. 1).

The etiological agent of CSF is classical swine fever virus (CSFV), which belongs to the genus *Pestivirus*, family *Flaviviridae*, together with bovine viral diarrhea virus (BVDV) and border disease virus (BDV) (Rice, 2001). Strategies to control CSFV mainly consist of a stamping out policy (non-vaccination) and a systematic prophylactic vaccination (see Edwards et al., 2000; Huang et al., 2014 for reviews). Under the non-vaccination policy, a CSFV outbreak always cause huge economic losses in areas with high-densities of pigs (see Dong and Chen, 2007 for a review). Therefore, many countries, except those in the European Union, use a systematic

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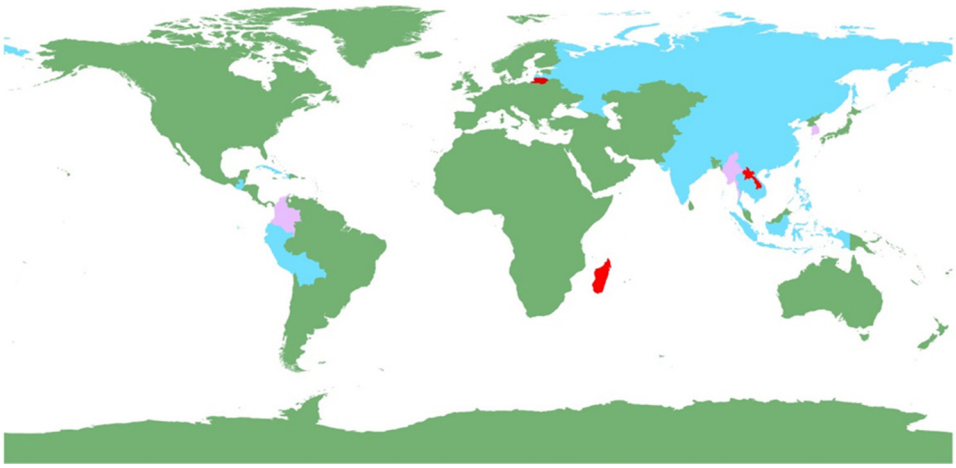

Fig. 1. Global distribution of CSF epidemics during 2011–2014 based on data from the OIE's new World Animal Health Information System (WAHIS). Four countries (red) reported CSF outbreaks in 2011–2012: Madagascar, Laos, Singapore and Lithuania. Three countries (lilac) reported CSF outbreaks in 2013–2014: Colombia, Myanmar and Rep. of Korea. Eighteen countries (blue) reported CSF outbreaks in both 2011–2012 and 2013–2014: Bolivia, Cuba, Ecuador, Guatemala, Haiti, Peru, Bhutan, Cambodia, China (People's Rep. of), India, Indonesia, Mongolia, Nepal, Philippines, Thailand, Vietnam, Latvia, and Russia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

prophylactic vaccination to control or eradicate CSFV (see Van Oirschot, 2003 for a review). For instance, compulsory vaccination is the current policy in China, and vaccination coverage must be over 90% at any time in the year in the swine population (reviewed in Luo et al., in press). Many vaccines were developed after the pathogenic agent of CSF was shown to be a virus in 1904, (see Dong and Chen, 2007 for a review). Modified live vaccines (attenuated CSFV strains produced through serial passage in non-natural hosts; Table 1) have predominately been used and the most common vaccine is the commercial C-strain, because it is more effective and safer than other vaccines, such as the subunit differentiating infected from vaccinated (DIVA) individuals vaccine (see Huang et al., 2014 for a review). The C-strain vaccine up-regulates the antibody level and it also activates T-cell responses, while the subunit E2 vaccine only induces production of an antibody against CSFV (Huang et al., 2014).

Phylogenetic analysis of CSFV strains suggests there are three genotypes (Postel et al., 2012) that can be divided into high virulence, moderate virulence, and low to avirulent strains (the main vaccine strains) (Leifer et al., 2011). The highly virulent and modified live vaccine strains that have been identified belong to genotype 1, while the genotype 2 and 3 strains have moderate and low virulence (Dong and Chen, 2007; Leifer et al., 2011). Genotype 1 CSFV can accumulate many mutations in the field, as demonstrated by our research (Ji et al., 2014) and CSFV outbreaks caused by genotype 2 have been increasing in Europe and Asia (see Huang et al., 2014 for a review). This indicates that subunit E2 vaccines derived from genotype 1 strains may lose their effectiveness against virulent CSFV derived from genotype 2. The C-strain vaccine may continue to be effective for a longer time, due to T-cell responses. The GPE-strain vaccine can obtain pathogenicity by passaging it in pigs (Tamura et al., 2012), when there are mutations and selection

Table 1
The modified live vaccine were produced through serial passage in non-natural hosts.

Modified live vaccine	Country	Original strain	Adapted host	Reference
LPC	China, Taiwan	ROVAC	Rabbit	Lin et al. (1974), Lin et al. (1981)
HPLC	China, Mainland	Shimen	Rabbit	Tc (1980), Zhou (1980)
ROVAC	American		Rabbit	Koprowski et al. (1946)
SFA	British		Rabbit	Baker (1946)
GPE-	Japan	ALD	Swine testicle, bovine testicle and guinea-pig kidney cells, (29–30 °C)	Sasahara et al. (1969), Shimizu et al. (1970)
Thrivel	French	Alfort	Swine cell, Bovine cell (29–30 °C)	LunaisM (1972)
LOM/(Flc-LOM)	Japan	Miyagi(Japna)	Bovine kidney cells	Nishimura et al. (1964), Park et al. (2012)
Pestiffa	French	C-strain	Lamb kidney cells	Terpstra (1992)
SUVAC	Hungary	C-strain	Sheep	Olah (1985), Terpstra, (1992)
Cellpest	Poland	C-strain	PK-15A cell line	Pejsak et al. (1991)
Suiferin C	Former East Germany	C-strain		Dong and Chen (2007)
ЛК	former USSR	C-strain		Dong and Chen (2007)
VADIMUN	USA	C-strain		Dong and Chen (2007)
Riems	Germany	C-strain		Liess (1988)
Duvaxin	Germany			Björklund et al. (1998)
Norden	Mexico			Björklund et al. (1998)
Pestipan				Björklund et al. (1998)
Porcivac	Mexico			Björklund et al. (1998)
PS Poreo	Brazil			Björklund et al. (1998)
Tipest	Slovakia			Björklund et al. (1998)
TVM-1	Czech Republic			Björklund et al. (1998)
Russian LK	Russia			Björklund et al. (1998)

The strain of swine fever virus for the manufacture of a vaccine against classical swine fever, http://www.findpatent.ru/patent/205/2057805.html.

on the CSFV genome from the quasispecies population. Although the C-strain could not revert to virulence through serial pig-to-pig transmission experiments (Bran et al., 1966; Liess, 1988; Lin et al., 1972), it can recombine with the field-form of the CSFV virus, engendering new strains with unknown characteristics (Ji et al., 2014). In response to the above research, fabrication of a DIVA-maker vaccine from genotype 2 is required for better control and eradication of CSFV, and this manufacture is dependent on the manipulation of reverse genetics systems and knowledge of CSFV protein functions.

2. Reverse genetics systems and study of CSFV Virulence

2.1. Reverse genetics systems

Reverse genetics (RG) is the creation of a virus with a full-length copy of the viral genome and is the most powerful tool in modern virology. The purpose of RG is to investigate the virus' replication, infection, the function of viral proteins, the incidence of recombination, virus interaction with the host, antivirus strategy and the development of maker vaccines. At least five methods for rescuing CSFV have been developed and have driven the progress of CSFV research. A landmark study reporting the details of an in vitro transcription system was published in 1996, where infectious CSFV was recovered from porcine cells (SK6 cell line) through transfection of in vitro-transcribed RNA derived from a cDNA construct (Fig. 2A; Meyers et al., 1996; Moormann et al., 1996). However titers of the in vitro-transcribed RNA were relatively low, and four better reverse genetic systems were subsequently developed: (I) Fan et al. (2009) authenticated that the transfected efficiency of BHK-21 is 10-fold higher than PK15 and constituted a new procedure using the high-efficiency transfected cells (BHK-21) to transfect in vitro synthesized RNA transcripts and porcine kidney cells (PK15)IX by assessing the transfected efficiency of BHK-21 (with no CSFV receptor) and the transfection efficiency of PK15 (with a CSFV receptor), and found that multiplication with a centrifugal supernatant of infected PK15 cells resulted in an 8-fold increase in defects compared to straightforward manipulations of RNA into PK15 (Fig. 2B). (II) Van Gennip et al. (1999) developed a novel, more convenient and more efficient, in vivo transcriptional system, with a 200-fold increase in virus titles compared to an in vitro transcriptional system by establishing a stable swine kidney cell line expressing T7 RNA polymerase (SK6-T7RNApol) and transfecting with a linearized plasmid DNA into SK6-T7RNApol (Fig. 2C). (III) Bergeron and Perreault (2002) inserted the Hepatitis delta virus ribozyme (Hdv RZ) to function as an enzyme to form a precise 3′NCR. The 3′NCR of the virus genome can circumvent linearization of template DNA with the aid of an expensive enzyme called *SrfI* (Zou et al., 2011). By avoiding linearization, Van Gennip et al. (1999) utilized SK6-T7RNA-pol for in vivo transcription with circular DNA, resulting in a 20 fold increase in virus titers compared to in vitro transcription, but a 10-fold decrease compared to in vivo transcription of linearized DNA. Huang et al. (2013) exploited the PK-15 cell line's ability to stably express T7 RNA polymerase (PK15-T7RNApol) to use it for in vivo transcription (Fig. 2D). (IV) Li et al. (2013a) constructed a CSFV cDNA clone flanked by a CMV promoter, intro, T7 promoter and a hammerhead ribozyme (HamRZ) at the 5′NCR, and flanked by HdvRZ, T7 terminator and SV40 at the 3′NCR (Li et al., 2013a) (Fig. 2E). With this engineered CSFV, HamRZ can form a precise 5′NCR (Haseloff and Gerlach, 1988) and this cDNA clone can be rescued by T7 RNA polymerase or the RNA polymerase II system (CMV promoter), with production of 120-fold more progeny virus titers from in vivo transcription by a CMV promoter than the in vitro transcription by the T7 promoter.

Some derivative cDNA clones were established for CSFV investigation. A recombinant CSFV stably expressing a chloramphenicol acetyltransferase (CAT) gene represents a useful tool for quantitative analysis of viral replication and gene expression. According to this alteration, Qiu's lab developed three analogous recombinant cDNAs: (I) EGFP-CSFV cDNA was generated by inserting the EGFP gene between amino acids 13 and 14 of the Npro protein of CSFV for a Simplified Serum Neutralization Test (Li et al., 2013b). However, EGFP detection requires extensive and costly automated imaging equipment and does not integrate with high-throughput screening (HTS) assays. To solve this problem, a reporter version of the CSFV-Npro Fluc virus was developed that stably expressed the firefly luciferase (Fluc) gene in the Npro gene for rapid and quantitative screening and evaluation of antivirals against CSFV (Shen et al., 2014). Additionally, a cDNA with a biarsenical tetracysteine (TC) tag (CCPGCC) in the N-terminal region of the Npro protein was constructed to visualize the nucleus import and export of the Npro protein (Li et al., 2014c). These visualized modifications can be used as maker vaccine candidates and are useful in studying CSFV in depth.

Hitherto many significant discoveries have depended on reverse genetics systems to establish a foundation for studying the mechanisms of virulence and the functions of CSFV proteins. These results have led to new maker vaccines through mutation, recombination or other changes to the CSFV genome (see Dong and Chen, 2007 for reviews).

2.2. Molecular basis of CSFV virulence

The alteration of virulence can easily be obtained by in vitro introduction of mutations in conserved domains related to virus entry and release, speed of replication, interaction with host cell protein, and other essential physiological processes (see Leifer et al., 2013 for a review). Thus far, seven proteins (Npro, Core, Erns, E1, E2, P7, NS4B) have been found to be related to virulence in CSFV through reverse genetic modification of cDNA. Deletion of Npro can decrease virulence and induce immunity of specific pathogen free (SPF) swine, suggesting Npro is related to the virulence to some extent (Mayer et al., 2004; Tamura et al., 2014). Four conserved domains of the Core protein (I, II, III, and IV) can interact with the IQGAP protein (an effector of Rac1 and Cdc42), and substitutions in the conserved domains I and III of Core protein, interact with the host cell protein IQGAP1, resulting in a complete absence of virulence (Briggs and Sacks, 2003; Gladue et al., 2011a; Watanabe et al., 2004). Replacement of the K11A, K12A, and K53A amino acids of the Core protein disrupts the interaction between Core and SUMO-1, and replacement of K220A precludes an association between Core and UBC9. All of these substitutions will attenuate CSFV (Gladue et al., 2010). Another way to attenuate CSFV is to destroy the glycosylation site of Erns by N2A/Q substitution (Sainz et al., 2008), suggesting that glycosylation is related to virulence. To date, all analyzed pestiviruses contain four intramolecular disulfide bonds that are formed by eight conserved cysteine residues of Erns (Tews et al., 2009). The intra-strand disulfide bonds are formed by a ninth cysteine residue (171Cys) that stabilizes the Erns homodimer and can influence virulence (Langedijk et al., 2002; Tews et al., 2009). CSFV can also be attenuated when 30His and 79His (79AA is located in a conserved domain of the RNase T2 family (Irie and Ohgi, 2001; Krey et al., 2012)) of Erns are deleted, due to the destruction of RNase activity (Hulst et al., 1998; Meyers et al., 1999). The high virulence strain Brescia could be completely attenuated by inserting 19 amino acids into the C terminal of E1, suggesting that the C terminal domain plays an important role in virulence (Risatti et al., 2005a). Specifically, the high virulence strain BICv can lose fertility and be attenuated when N6A, N19A, and N100A amino acids of E1 are substituted (Fernandez-Sainz et al., 2009). The highly virulent strain

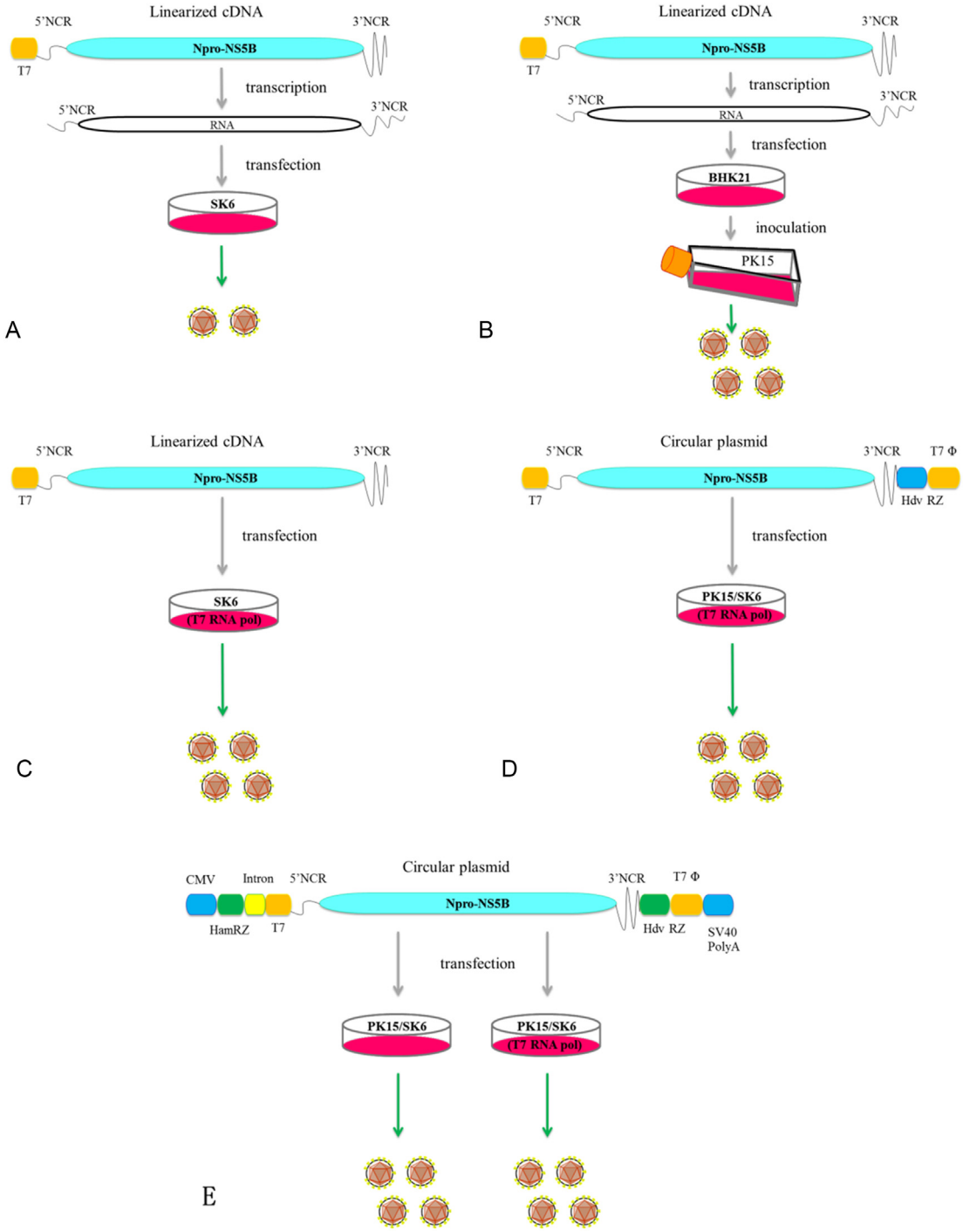

Fig. 2. Five reverse genetics systems for rescuing CSFV. (A) Infectious CSFV was recovered from porcine cells (SK6 cell line) through transfection of RNA derived from a linearized cDNA transcribed in vitro by T7 RNA polymerase. (B) The in vitro-transcribed RNA were used to transfect a high transfection efficacy hamster cell line (BHK-21) and the supernatant from the BHK21 cell-culture medium was used to infect the PK15 cell line to rescue virons. (C) A stable swine kidney cell line expressing T7 RNA polymerase (SK6-T7RNApol) was established for in vivo transcription of a linearized cDNA. (D) The circular vector can be used for in vivo transcription via insertion of Hdv RZ after the 3′NCR of the viral genome and establishment of a PK-15/SK6 cell line that consistently expresses T7 RNA polymerase. (E) Construction of a CSFV cDNA clone flanked by the CMV promoter, intron, T7 promoter and HamRZ at 5′NCR, and by HdvRZ, T7 terminator and SV40 at 3′NCR can be used to rescue CSFV by T7 RNA polymerase or the RNA polymerase II system (with a CMV promoter).

Brescia can also be attenuated when the E2 gene is replaced, suggesting that E2 is the primary determinant of virulence (Risatti et al., 2005b). The conserved epitope 140-TAVSPTTLR-148 located on the A domain of the E2 protein plays an important role in virulence (Risatti et al., 2006). The E2 gene has an O-linked glycosylation site and six N-linked glycosylation sites, which all influence virulence and also play a role in the production of swine protective antibodies and the subunit vaccine (Gavrilov et al., 2011; Risatti et al., 2007a). A mutation of 710Leu → His in E2 will attenuate the virus only if there are additional mutations on Erns (276R, 476R, 477I; (Van Gennip et al., 2004). Replacements of groups of amino acids in the C terminal of the E2 protein can also influence the virulence of CSFV (Risatti et al., 2007b). P7 can influence CSFV virulence, replication and also the formation of infectious virions (Gladue et al., 2012;

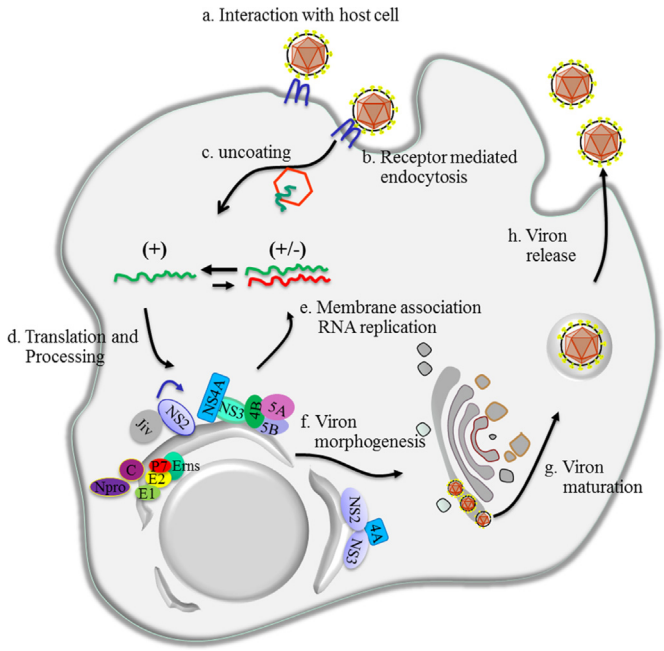

Fig. 3. Schematic diagram of the CSFV life cycle. The life cycle of CSFV is similar to the life cycles of other members of the *Flaviviridae* family. Extracellular CSFV virions encounter and interact with receptor molecules at the cell surface (a) and go through receptor-mediated endocytosis (b) into a low-pH vesicle, which triggers conformational changes in the virion, fusion of the viral and cell membranes, particle disassembly, and the release of viral RNA into the cytoplasm (c). The genomic RNA is translated to a single large polyprotein that is then processed into 12 mature proteins in association with the ER membrane (d). The mature proteins replicate the RNA genome via a minus-strand replicative intermediate to produce progeny positive-strand RNA: a fraction of this newly synthesized RNA is packaged into nucleocapsids for budding into the ER (f). Virons follow the cellular secretory pathway and, during this transportation, maturation of infectious particles occurs (g). Mature virons are released from the cell, completing the life cycle (h). + Is positive-sense genomic RNA; ± is minus-strand replicative intermediate associated with positive-strand genomic RNA.

Harada et al., 2000). There is a putative Toll/interleukin-1 receptor (TIR)-like domain in NS4B that consists of two conserved box1 (195IYKTYLSIRR204) and box2 (228SVGIAVML235) domains and mutations in these conserved domains can influence the virulence of CSFV (Fernandez-Sainz et al., 2010). On the basis of these investigations, we estimate that artificial modification in the conserved domains will lead to alteration of virulence of CSFV. Moreover, the virulence of CSFV might depend on multiple mechanisms involving Npro, C, Erns, E2, P7, and NS4B.

3. CSFV life cycle and molecular features of the CSFV genome

3.1. CSFV life cycle

In recent years significant progress has been made in understanding key steps of the CSFV life cycle, although many steps remain enigmatic (Fig. 3). Once CSFV reaches the host target cell, the infection cycle begins with the attachment and entry process, which is mediated by the E1 and E2 glycoproteins (Wang et al., 2004), while the cell surface receptor is undefined (Gladue et al., 2014b) (possibly CD46, the receptor of BVDV (El Omari et al., 2013)), and the mechanism of CSFV endocytosis is still unclear (BVDV entry is dependent on clathrin-mediated endocytosis (Lecot et al., 2005)). After CSFV entry, the membrane fusion is mediated by glycoproteins in a pH-dependent way, triggered by acidification (Sieczkarski and Whittaker, 2002; Wang et al., 2004) and the peptide ^{129}CPIGWTGVIEC^{139} in E2 may be involved in membrane fusion (Fernández-Sainz et al., 2014). After fusion of the viral envelope and the cell plasma membrane, the viral nucleocapsid is released into the cytosol of infected cells where cap-independent translation gives rise to a polyprotein and replication of the viral genome is initiated. CSFV replication requires the synthesis of a complementary RNA(−), which is used as a template for further synthesis of progeny RNA(+), based on the observation that the NS5B protein binds the minus-strand 3′ UTR more efficiently than the plus-strand 3′ UTR (Xiao et al., 2004). Then NS2–3, along with cofactor NS4A, mediates viron morphogenesis (Moulin et al., 2007). Finally, virions are released from the host cell following maturation of the infectious particles (David and Knipe, 2013b). All mature proteins (except Npro) and the precursor NS2–3, are essential to the life cycle of CSFV, while NS3, NS4A, NS4B, NS5A, NS5B are required for CSFV RNA replication (Moulin et al., 2007). Upon infection, several biological processes of the infected cells are modulated to enhance their replication and to some extent mediate immune evasion. These are regulation of the SUMOylation pathway by Core, interaction between actin and E2, induction of S phase retardation by NS2, stimulation of autophagy by NS5A and modulation of innate immunity by Npro and Erns (Fig. 5).

3.2. Molecular features of CSFV genome and protein

CSFV is a single-stranded, non-segmented, positive-sense RNA virus, and the diameter of this lipid-enveloped RNA virus is approximately 40–60 nm. The genome consists of a non-capped 5′ UTR (containing the internal ribosome entry site or IRES), an open reading frame (ORF), and a 3′ UTR lacking a poly A region (Thiel et al., 1991). The ORF encodes a 3898 amino acid polyprotein that undergoes transcriptional and post-transcriptional modifications by a signal peptidase from the host cells and by Npro, NS2, and NS3 proteinase from CSFV (Bintintan and Meyers, 2010; Gottipati et al., 2013; Heimann et al., 2006; Moulin et al., 2007; Rümenapf et al., 1998; Tang et al., 2010). Two precursor proteins and 12 mature proteins are produced from the polyprotein: four structural proteins (C, Erns, E1, E2), eight non-structural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) and two precursor proteins (E2-P7, NS2–3; Fig. 4A). The precise cleavage sites between adjacent proteins are shown in the entry for NC_002657 of the NCBI-Viral Genomes database (Bao et al., 2004). We defined the protein topologies using bioinformatics, biochemical analysis and previous research on CSFV (Fig. 4B).

The uracil-rich 3′ UTR is approximately 232 bp and is involved in the initiation of virus replication (Björklund et al., 1998). The 3′ UTR consists of two NS5A binding sites: 3′ UTRSL-1 and 3′ UTRSL-2. When NS5A is present in low concentrations, it joins with NS5B to preferentially bind to 3′ UTRSL-1. When NS5A is present in high concentrations, it may also bind to 3′ UTRSL-2 in order to inhibit RNA replication (Sheng et al., 2012). The Sheng et al. (2012) study also indicated that NS5A has a higher affinity to the 3′ UTR than to NS5B and NS5A has a higher affinity to 3′ UTRSL-1 than to 3′ UTRSL-2. Previous research demonstrated that the 3′NCR insertion of 13 nucleotides in lapinized vaccine strains might be incorporated during the adaptation of the virus to the rabbit host system (Björklund et al., 1998). Li's research (2014a) subverted this hypothesis and demonstrated that the noncoding regions (NCRs) of C-strain were essential for inducing fever in rabbits, but not necessary for viral replication and adaptation in the spleen of rabbits.

The 5′ UTR region of CSFV is approximately 375 bp, and initiates cap-independent translation (Fletcher and Jackson, 2002; Zhu et al., 2010). The IRES is located between 60 nt and 375 nt in the 5′ end and can be recognized by the ribosome to initiate translation. This initiation process is similar to the initiation of translation in prokaryotes in that the ribosome binds directly to the Shine–Dalgarno (SD) sequence (Fletcher et al., 2002). Virus translation is regulated by

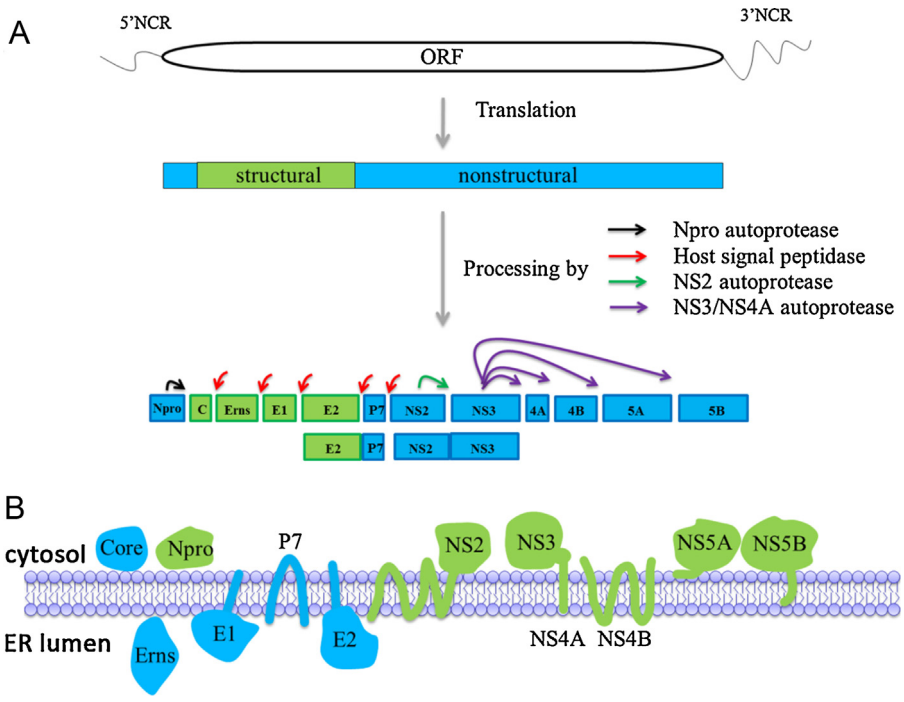

Fig. 4. Organization of the CSFV genome, polyprotein processing, and protein topology. (A) The CSFV genome is a single-stranded, non-segmented RNA, consists of a non-capped 5′ UTR (containing IRES), an open reading frame (ORF), and a 3′ UTR lacking a poly A region. The ORF encodes a 3898-amino acid polyprotein that undergoes a complex co- and post-translational series of cleavage events, catalyzed by both host and viral proteases, to produce 12 mature proteins and two precursor proteins. (B) The topology of CSFV proteins relative to the ER membrane.

the IRES and viral genomes generally have a long 5′ UTR with multiple AUG codons (Hellen and Sarnow, 2001). The IRES of CSFV contains complete domains II and III, stems 1 and 2, and a loop between the two stems (Fletcher and Jackson, 2002). The deletion of domain II destroys the function of IRES, stem mutations substantially decrease the activity of IRES, and the size of the loop is important for proper IRES function (Fletcher and Jackson, 2002). A study by Zhu et al. (2010) found that IRES contains the binding sites for NS3 and NS5A, which may participate in both RNA replication and translation. IRES mediation of RNA translation, as well as replication, has also been illustrated in hepatitis C virus (HCV) (Friebe et al., 2001).

The Npro gene codes for a 19 kDa protein of approximately 168 amino acids (Ruggli et al., 2005). Npro is a cysteine protease (Glu22-His49-Cys69 is the active center) (Rümenapf et al., 1998) that forms its own C53 cysteine protease family (http://merops.sanger.ac.uk/) (Rawlings et al., 2014). Npro has an N terminal catalytic domain and a C terminal zinc-binding domain (a metal-binding TRASH domain consisting of C112-X21-C134-X3-C138) (Gottipati et al., 2013; Szymanski et al., 2009). The *cis*-protease activity of Npro results in the release of Npro from a polyprotein (Rümenapf et al., 1998). The complete non-virus protein was able to be produced when *eGFP* and a foot-and-mouth disease virus 2a protease gene (FMDV *2apro*) were inserted between *npro* and *core* (Fan and Bird, 2008), demonstrating the *cis*-protease activity of Npro. Replacement of Npro by a murine ubiquitin gene showed growth characteristics similar to those of the parent virus (Tratschin et al., 1998), indicating that Npro is not required for viral replication. Structural analysis of the released Npro shows no protease activity because the C terminal domain blocks the N terminal catalytic domain (Gottipati et al., 2013). Npro works in tandem with the C terminal zinc-binding domain to decrease the levels of IFN regulatory factor 3 (IRF3) and IFN regulatory factor 7 (IRF7) through a ubiquitin proteasome pathway and the reduction of IRF3 and IRF7 can inhibit the production of type I interferon and then decrease the anti-virus reaction in cells (Bauhofer et al., 2007; Chen et al., 2007; Fiebach et al., 2011; Gil et al., 2006; Iwasaki and Medzhitov, 2010; Ruggli et al., 2003; Seago et al., 2007; Szymanski et al., 2009; Tarradas et al., 2014). Npro also plays an important role in the process of escaping RNA-induced apoptosis through interactions with HAX-1 (Johns et al., 2010a,b). Genes in the HAX-1 and Bcl-2 families possess the homologous domain BH1, a potent inhibitor of Bax-induced apoptosis (Sharp et al., 2002). This process promotes cell survival by inhibiting the activation of caspase9 (Han et al., 2006). However, the double-stranded RNA formed by the 5′ and 3′ UTRs of CSFV that triggers cell apoptosis cannot be suppressed by Npro (Hsu et al., 2014).

The Core gene codes for a 14.3 kDa protein with 99 amino acids, which is rich in alkaline amino acids (lysine and arginine) and influences the host gene expression (Heimann et al., 2006; Liu et al., 1998). The Core protein is released from a polyprotein after the N terminal is cleaved by Npro and the C terminal is cleaved by a host signal peptidase (Gottipati et al., 2013; Heimann et al., 2006). Cleavage inhibition of Npro leads to the accumulation of Npro-core precursor proteins in the host cells (Rümenapf et al., 1998), which clarify cleavage activity of Npro. Deletion experiments have shown that 11–12AA, 26–30AA, and 40–44AA of the Core protein are of vital importance to the production of progeny viruses (Riedel et al., 2010). Deletion of 1–77AA of the Core protein leads to infertility, which is reversible by providing the Core protein in *trans*, and Ala87 seems to play an especially important role in fertility (Riedel et al., 2010). The Core protein can enhance CSFV RNA replication in a virus species-specific manner (Li et al., 2014b), and substitution of the OS9 binding site significantly decreases replication efficacy (Gladue et al., 2014a). In addition, the Core protein can be involved in the SUMOylation pathway by interacting with SUMO-1 (small ubiquitin-like modifier) and UBC9 (SUMO conjugating enzyme), which eventually mediates virus proliferation (Gladue et al., 2010) and immune evasion of CSFV. This has also been shown in some other viruses, such as the human cytomegalovirus (Huh et al., 2008), Epstein-Barr virus (Shirai and Mizuta, 2008), dengue

virus (Chiu et al., 2007), Moloney murine leukemia virus, (Yueh et al., 2006) and vaccinia virus (Palacios et al., 2005).

Erns is a shortened form of "envelope protein RNase secreted" and codes for a 44 kDa protein consisting of 227 amino acids (van Gennip et al., 2000). A cleavage site between Erns and E1 is processed by the host signal peptidase and this process takes place in the ER lumen (Bintintan and Meyers, 2010). Erns creates disulfide links to form a 100 kDa highly glycosylated homo-dimer in which N-linked glycosylated residues account for half of the molecular mass (Branza-Nichita et al., 2004; Langedijk et al., 2002). Erns can attach to the cell surface of various species (Hulst and Moormann, 1997) even if those species are not susceptible to CSFV, since the translocation of Erns is energy-independent and not mediated by a protein receptor (Langedijk, 2002). Erns induces immune responses as a minor neutralizing antigen during infection (Weiland et al., 1992). Erns has endonuclease activity and contains two of the universal active domains of the RNase T2 family (CSFV: 38SLHGIWPG45 and 75EWNKHGWC82) (Irie and Ohgi, 2001; Krey et al., 2012) and tends to cleave the linkage of NpU (Hausmann et al., 2004). Erns can regulate the synthesis of RNA in host cells, inhibit protein synthesis, induce apoptosis of lymphocytes and inhibit early-stage immunization of the host (Bruschke et al., 1997; Hulst et al., 1998). Erns has no transmembrane domain, and can be secreted out of the host cells after maturation (Rümenapf et al., 1993). Studies on Erns in BVDV have shown that Erns can be transported to other parts of the body through the circulatory system and inhibit the activation of the Toll-like Receptor (TLR) by degrading extracellular virus ssRNA or dsRNA that can inhibit the production of type I interferons and assist in the coexistence of the virus and its host (Mätzener et al., 2009; Magkouras et al., 2008). It is possible that the Erns protein in CSFV functions in the same pathways, but this remains to be tested.

The E1 gene codes for a 33 kDa protein consisting of 195 amino acids. E1 is a type I transmembrane protein (Weiland et al., 1990). The N terminal of E1 is an ectodomain and the C terminal is a hydrophobic anchor that attaches E1 to the envelope of the virus (Thiel et al., 1991). E1 and E2 form heterodimers located on the envelope and mediate virus attachment and invasion (El Omari et al., 2013; Wang et al., 2004).

The E2-P7 gene codes for a 443 amino acid non-structural protein, which is the outcome of low efficient cleavage in the division site between the E2 and P7 proteins (Elbers et al., 1996). BVDV studies have shown that introducing mutations in the cleavage site causes the failure of E2 and P7 protein formation with normal RNA replication and flunked formation of infectious particle. This can be reversed by furnishing P7 and E2 in trans. The insertion of a stop codon in the signal peptide coding sequence and IRES between E2 and P7 brings about failed formation of the E2-P7 protein, yet infectious virions can still survive (Harada et al., 2000). These studies demonstrate that E2-P7 is not essential for the generation of infectious virions. The protein may have other functions, but further research is needed to discover these functions.

The E2 gene codes a 55 kDa structural protein that consists of 373 amino acids and has glycosylation on the surface and functions as a primary neutralizing antigen. Incision between E1 and E2 is executed by the host signal peptidase located in the ER lumen (Bintintan and Meyers, 2010). E2 is a type I transmembrane protein with a transmembrane domain in its C terminal that can anchor in the viral envelope (Li et al., 2013c; Risatti et al., 2007a). The N terminal is an extracellular motif containing four antigen domains: A, B, C, and D (Van Rijn et al., 1994) and the A domain has three subdomains: A1, A2, and A3 (Van Rijn et al., 1993). E2 can form E2–E2 homodimers (100 kD) or E1–E2 heterodimers (75 kD) with a disulfide bond (Weiland et al., 1990; Wensvoort et al., 1990). E2 (186–261AA, 262–341AA) can interact with β-actin (95–188AA) and influence viral replication and CSFV invasion, but glycosylation has no influence on these interactions (Fig. 5) (He et al., 2013). In addition, Gladue's study of a yeast two-hybrid system found that proteins from swine cells could interact with CSFV E2 proteins (Gladue et al., 2014b). This provides candidates of CSFV receptors, but further investigation is needed.

P7 is a 70 kDa two-transmembrane protein that consists of 70 amino acids (Elbers et al., 1996). The P7 protein forms an ionic channel that consists of two hydrophobic domains (TM1 and TM2) and a cytosolic loop linking the two domains, and both the N terminal and C terminal domains of P7 are positioned toward the ER lumen (Guo et al., 2013a). Cleavage sites between E2/P7 and P7/NS2 are located in the N terminal of N7 and NS2 and can be cleaved by host signal peptidase (Bintintan and Meyers, 2010; Elbers et al., 1996; Harada et al., 2000). These cleavage sites rely on microsomal membranes, suggesting that the late stage process of polyprotein formation occurs in the ER (Elbers et al., 1996; Guo et al., 2013a). At an early stage of viral replication, P7 attaches to the ER membrane by co-translation and the polarity sequence RDEPIKK that is positioned in the cytosolic region may initiate budding process and mediate virus packaging (Elbers et al., 1996). P7 can influence CSFV replication and the formation of infectious virions (Gladue et al., 2012; Harada et al., 2000). Furthermore, p7 protein is a short-lived protein degraded by the proteasome that induces the proinflammatory cytokine IL-1β secretion, which is one of the fundamental reactions of the innate immune response to viral infection (Lin et al., 2014).

NS2–3 is a gene that codes for a 120 kDa protein consisting of 1140 amino acids. NS2–3 recruits NS4A to form an NS2–3–4A complex used to produce infectious virions (Lamp et al., 2013; Moulin et al., 2007). Studies of BVDV indicate that the insertion of ubiquitin or IRES between NS2 and NS3 can inhibit formation of the NS2–3 complex. This has no effect on RNA replication, but halts the production of infectious virions. Virion production can be regained by co-expressing NS2–3 (Agapov et al., 2004). NS2–3 recruits NS4A and together they recruit NS4B, NS5A and NS5B (Moulin et al., 2007). At this point, NS2 binds to JIV with a *cis*-cleavage release of NS2, and then an RNA replication complex is formed by NS3, NS4A, NS4B, NS5A and NS5B (Lackner et al., 2006; Moulin et al., 2007). If NS2–3 succeeds in separating, then RNA replication will occur. If NS2–3 fails to separate, then formation of infectious particles will be mediated by interactions with NS4A (Moulin et al., 2007).

NS2 contains 457 amino acids and exhibits auto-protease activity (Lackner et al., 2006). NS2 can be released from NS2–3 by *cis*-cleavage and is fixed to the ER by highly hydrophobic domains in the N terminal (Tang et al., 2010). NS2 can accelerate the proteasomal degradation of Cyclin A, but also increase the transcriptional levels of Cyclin A (Tang et al., 2010). When this occurs, the host cell suspends growth in the S phase and creates a good environment for virus RNA replication (Tang et al., 2010). This phenomenon has also been found in HCV (Yang et al., 2006), herpes virus (Kudoh et al., 2003) and human T-lymphotrophic virus type I (Liang et al., 2002) infected host cells. In vitro experiments indicated that NS2 is not essential to RNA replication, but could prolong the half-time of replication in transfected cells (Moser et al., 1999). NS2 expression can result in an ER-stress response (Tang et al., 2010) and the activation of NF-κB, which can lead to the up-regulation of IL-8 (Tang et al., 2011), which can antagonize type I IFN response (David and Knipe, 2013a). The anti-apoptosis factor Bcl-2 can be up-regulated to inhibit MG132-induced apoptosis by the NS2 protein (Tang et al., 2011). In addition, phosphorylation of short-lived NS2 by the protein kinase CKII can promote the degradation of NS2 (a reaction that is otherwise inhibited by MG132) (Guo et al., 2013b).

NS3 codes for a 80 kDa protein consisting of 683 amino acids (Lamp et al., 2013). This is a multi-functional protein that exhibits serine protease, RNA helicase, and NTPase activities and plays an important role in transcription and translation (Sheng et al., 2007).

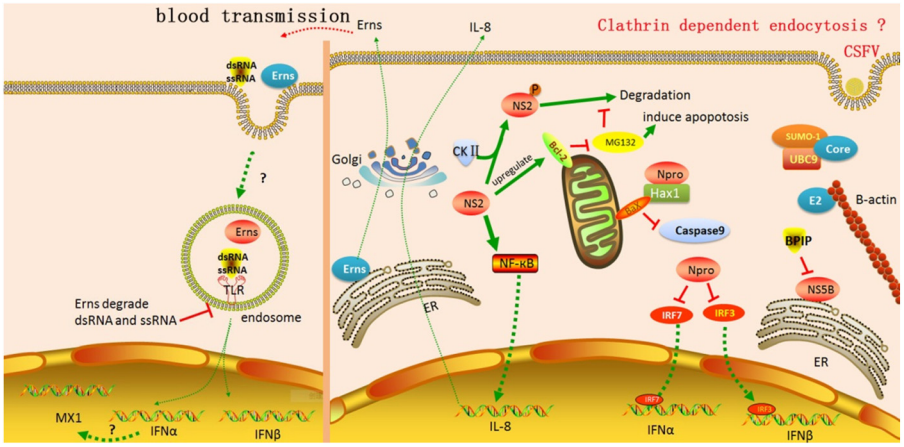

Fig. 5. Interaction pathways between CSFV and host cells. (I) Npro plays an important role in the process of escaping dsRNA-induced apoptosis through an interaction with HAX1, which inhibits Bax-induced apoptosis and then promotes cell survival by inhibiting the activation of caspase9. Npro can decrease the content of IRF3 and IRF7 through the ubiquitin proteasome pathway that ultimately results in inhibiting the production of type I interferon and then interfering with anti-virus reactions in the cells. (II) Core proteins play a role in the SUMOylation pathway via interaction with SUMO-1 and UBC9 in order to mediate virus proliferation and evasion. (III) Erns may be transported to other cells by the blood and inhibit the activation of Toll-like Receptors (TLR) by degrading the extracellular virus ssRNA or dsRNA, that leads to the inhibition of type I interferon production and allows the virus and host to coexist (Mätzener et al., 2009; Magkouras et al., 2008). (IV) E2 can interact with β-actin. (V) NS2 protein can up-regulate the expression of the anti-apoptosis factor Bcl-2 to inhibit the MG132 induced apoptosis and this allows CSFV to coexist with the host cell. Phosphorylation of NS2 by protein kinase CK II can promote the degradation of NS2 that can be inhibited by MG132. NS2 expression leads to the activation of NF-κB and can up-regulate the expression of IL-8. (VI) BPIP can suspend the replication and diffusion of CSFV via NS5B and may provide a new target for anti-virus studies. (VII) IFN-α may up-regulate expression of MX1 to inhibit the replication of CSFV.

The serine protease domain is situated in the first third of the N terminal sequence and the RNA helicase and NTPase domains are located in the C terminal (Xu et al., 1997). It has been shown that the three domains rely on each other for proper functioning (Voigt et al., 2007). NS3 contains a nucleotide binding domain (NBM) found in all helicases and most NTPases (Gorbalenya and Koonin, 1989a; Walker et al., 1982). The NS3 helicase relies on NTP and a bivalent cation (especially ATP and Mn^{2+}) and utilizes the 3′ unpaired RNA as a template (Wen et al., 2007). NS3 serine protease can *cis*-cleave NS3–4A to release NS3 and can *trans*-cleave NS4A, NS4B, NS5A and NS5B, relying on a 64 amino acid cofactor in NS4A (Lamp et al., 2013; Moulin et al., 2007; Tautz et al., 1997; Tautz et al., 2000; Xu et al., 1997). NS3 serine protease can increase helicase activity, but has no influence on NTPase (Wen et al., 2009). The helicase activity of NS3 can promote the translation activity mediated by IRES (Zhu et al., 2010). A bioinformatic analysis showed that the RNA helicase in NS3 has a classic DEYH motif found in superfamily II helicases and mutations in the DEYH motif influences RNA replication (Sheng et al., 2010). NS3 helicase can promote IRES-mediated translation and cell-mediated translation (Xiao et al., 2008). The accumulation of NS3 in cells can result in cytopathy (Aoki et al., 2004).

NS4A consists of 64 amino acids and is a cofactor of NS3 serine protease. This protein influences RNA replication and the formation of infectious virion (Moulin et al., 2007). NS4B consists of 347 amino acids and exhibits NTPase activity. There are two conserved domains: WalkerA (209–216AA) and WalkerB (335–342AA) (Gladue et al., 2011b). In vitro expression of both wild type and mutant NS4B indicates that WalkerA is the key site for NTPase activity and is essential for RNA replication (Gladue et al., 2011b).

The NS5A protein consists of 497 amino acids. NS5A can stimulate the assembling of polyproteins and inhibit transcription and translation (Sheng et al., 2010; Xiao et al., 2009). The conserved domain (C2717–C2740–C2742–C2767) forming the zinc-binding motif is of vital importance to RNA replication and virus growth (Sheng et al., 2010). Studies of CSFV, HCV and BVDV found that expression of NS5A in trans can rescue RNA replication after NS5A deletion or mutation (Appel et al., 2005; Grassmann et al., 2001; Sheng et al., 2010), indicating that NS5A is a trans factor during replication. However, NS3 and NS5B deletions could not be rescued by supplementation, suggesting that NS3 and NS5B are *cis*-acting elements (Sheng et al., 2010). RNA replication that is influenced by NS5A deletions cannot be rescued by supplementation if there is a mutation in the N terminal amphipathic alpha-helix (Brass et al., 2002). NS5A can induce the autophagy pathway of host cells, which can enhance viral replication and the time until maturity of CSFV (Pei et al., 2013) and is a common effect in other viruses, such as the herpes simplex virus type 1 (Orvedahl et al., 2007), human immunodeficiency virus type 1 (Alirezaei et al., 2008; Kyei et al., 2009), coxsackie virus (Wong et al., 2008), dengue virus (Lee et al., 2008), influenza A virus (Zhou et al., 2009), and hepatitis C virus (Dreux et al., 2009). Mutation studies indicate that NS5B inevitably bound to 137–172 and 224–268 amino acids and could bind to 390–414 amino acids of NS5A (Chen et al., 2012). When NS5A is present at low concentrations, it prefers to bind to NS5B and promotes RNA replication. Oversaturated NS5A (137–172AA and 224–268AA) will bind to the 3′ UTR to inhibit RNA replication (Chen et al., 2012). Oversaturated NS5A can inhibit translation because the 390–414AA in the C terminal of NS5A can interact with IRES 60–130 nt and 234–393 nt to competitively inhibit the interaction of NS3 and IRES (Chen et al., 2012; Xiao et al., 2009; Zhu et al., 2010). NS5A can also inhibit translation due to the interactions between NS5A and NS3 (Lai et al., 2008). Altogether, NS5A can influence both RNA replication and translation. Furthermore, NS5A can induce the autophagy of host cells, which can enhance viral replication and maturity of CSFV in host cells, and may be related to immune evasion (Pei et al., 2013).

NS5B is an RNA-dependent RNA polymerase (RdRp) consisting of 718 amino acids that bind to a homologous sequence of the 3′ UTR to initiate translation (Xiao et al., 2002, 2004, 2006). Replicases (RdRp) can recognize and bind to the 3′ UTR of the positive strand RNA and produce negative strand RNA that can be used for the production of positive strand RNA (Gong et al., 1996). NS5B prefers to combine with the negative strand RNA to produce more positive strand RNA (in the CSFV genome) (Xiao et al., 2004). Almost all RdRps have a GDD (Gly-Asp-Asp) conserved domain that serves as the active site for catalysis activity (Wang et al., 2007). A crystal structure analysis found that RdRps are comprised of five motifs, A to E (Bressanelli et al., 1999; Choi et al., 2004; Lai et al., 1999).

The A, B, C, and D motifs are Palm domains and the C motif forms a β-strand-turn-β-strand structure that contains the GDD conserved domain for ion regulation (Lai et al., 1999; Lohmann et al., 1997; Wang et al., 2007). Deletion studies indicate that NS3 is a serine protease (30–70AA) that can bind to the surface of NS5B (63–99AA, 611–642AA) and activate the RdRp (Wang et al., 2010, 2011). NS5B can stimulate NS3 to increase the rate of translation (Xiao et al., 2008). Surprisingly, NS5B can also interact with the protease domain of NS3 to weaken interactions between NS3 and IRES (Zhu et al., 2010). Based on these contradictory interactions, we have hypothesized the following sequence of events in the pathway: NS5B binds to the protease (30–70AA) of NS3. The protease then activates the helicase domain. The helicase further activates IRES mediated translation. When NS5B constantly binds to NS3, this accelerated translation will be inhibited. Furthermore, Xiao et al. (2004) found that NS5B exhibits nucleotidyl transferase activity.

Recent research has provided new and interesting insights into the functions of CSFV proteins and how they interact with the host cell at the molecular level, which in turn is offering hope for new strategies to prevent CSFV infection.

3.3. Antivirus

New developments in CSFV research may result in the discovery of new anti-virus targets. The fusion of CSFV capsid proteins and *Staphylococcus aureus* nuclease can specifically hydrolyze CSFV nucleotides. This technology is called capsid-targeted viral inactivation (CTVI) and has been developed for HIV-1, HBV, MLV, and dengue virus, and it might also be a valuable therapeutic approach against CSFV infection. Core proteins can activate the human heat shock protein 70 promoter and also inhibits the SV40 early promoter, suggesting that the core protein functions as a transcription factor for the regulation of gene expression and is a factor in the viral maturation process (Liu et al., 1998). RNA interference (specific to the core gene siRNA_253) can de-regulate virus duplication and viral gene expression (Porntrakulpipat et al., 2010). An RNAi strategy has also been used for other viruses, such as foot-and-mouth disease (Chen et al., 2004), influenza (Ge et al., 2003), porcine reproductive and respiratory syndrome (He et al., 2007) and porcine transmissible gastroenteritis (Zhou et al., 2007). The phage-displayed E2-binding peptides DRATSSNA can inhibit CSFV replication in PK-15 cells, which has the potential to be developed as antivirals for CSF (Yin et al., 2014). Two pore-forming inhibitors, amantadine and verapamil, were able to reduce the titer of CSFV, without affecting the viability of the host cell SK6 according to an MTT experiment (Gladue et al., 2012). BPIP (5-[(4-bromophenyl) methyl]-2-phenyl-5*H*-imidazo[4,5-c]pyridine) was able to suspend the replication and diffusion of CSFV via NS5B and this has provided a new method for the study of anti-virus methods (Haegeman et al., 2013; Vrancken et al., 2008, 2009a). Human MxA protein and Porcine Mx1protein can inhibit the replication of CSFV (He et al., 2014; Zhao et al., 2011) and IFN-α may up-regulate MX1 expression (Magkouras et al., 2008). Antiviruses could potentially target the regulation of Mx1. These ideas for antiviral research might transform CSFV treatment, but need more in-depth study.

4. Outlook

Despite recent progress in our understanding of CSFV, many questions remain. Understanding the mechanism of CSFV entry is a priority for controlling viral transmission. Though much is known about viral replication and translation, our understanding of the mechanism of virus proteins is still in its infancy. What determines the fate of newly synthesized RNA? What are the steps of virus assembly? Where does virus assembly occur and how do they release from the cell? Beyond multiple immune escape pathways, what other virus–host interactions are important for this clever RNA virus to establish and maintain persistent infection and outwit the supposedly sophisticated immune system of the host? An efficient commercial DIVA maker vaccine is required to eradicate CSFV. Antivirus strategies for controlling CSFV are at a preliminary stage of development and further research should be done to develop new commercial treatments.

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References

Agapov, E.V., Murray, C.L., Frolov, I., Qu, L., Myers, T.M., Rice, C.M., 2004. Uncleaved NS2–3 is required for production of infectious bovine viral diarrhea virus. J. Virol. 78 (5), 2414–2425.
U. Agriculture, 2010. UK Agriculture Livestock Classical Swine Fever.
Alirezaei, M., Kiosses, W.B., Flynn, C.T., Brady, N.R., Fox, H.S., 2008. Disruption of neuronal autophagy by infected microglia results in neurodegeneration. PLoS One 3 (8), e2906.
Aoki, H., Sakoda, Y., Nakamura, S., Suzuki, S., Fukusho, A., 2004. Cytopathogenicity of classical swine fever viruses that do not show the exaltation of Newcastle disease virus is associated with accumulation of NS3 in serum-free cultured cell lines. J. Vet. Med. Sci. 66 (2), 161–167 (the Japanese Society of Veterinary Science).
Appel, N., Herian, U., Bartenschlager, R., 2005. Efficient rescue of hepatitis C virus RNA replication by trans-complementation with nonstructural protein 5A. J. Virol. 79 (2), 896–909.
Baker, J.A., 1946. Serial passage of hog cholera virus in rabbits. Proc. Soc. Exp. Biol. Med. 63, 183–187 (Society for Experimental Biology and Medicine (New York, NY)).
Bao, Y., Federhen, S., Leipe, D., Pham, V., Resenchuk, S., Rozanov, M., Tatusov, R., Tatusova, T., 2004. National center for biotechnology information viral genomes project. J. Virol. 78 (14), 7291–7298.
Bauhofer, O., Summerfield, A., Sakoda, Y., Tratschin, J.-D., Hofmann, M.A., Ruggli, N., 2007. Classical swine fever virus Npro interacts with interferon regulatory factor 3 and induces its proteasomal degradation. J. Virol. 81 (7), 3087–3096.
Bergeron, L.J., Perreault, J.P., 2002. Development and comparison of procedures for the selection of delta ribozyme cleavage sites within the hepatitis B virus. Nucleic Acids Res. 30 (21), 4682–4691.
Bintintan, I., Meyers, G., 2010. A new type of signal peptidase cleavage site identified in an RNA virus polyprotein. J. Biol. Chem. 285 (12), 8572–8584.
Björklund, H., Stadejek, T., Belák, S., 1998. Molecular characterization of the 3′ non-coding region of classical swine fever virus vaccine strains. Virus Genes 16 (3), 307–312.
Bran, L., Mihaita, S., Popa, M., Totorcea, N., Albu, T., 1966. Sur la stabilité de quelques caractères biologiques de la souche C de virus lapinisé de la peste porcine. Bull. Off. Int. Epiz. 66, 681–693.
Branza-Nichita, N., Lazar, C., Dwek, R.A., Zitzmann, N., 2004. Role of *N*-glycan trimming in the folding and secretion of the pestivirus protein Erns. Biochem. Biophys. Res. Commun. 319 (2), 655–662.
Brass, V., Bieck, E., Montserret, R., Wolk, B., Hellings, J.A., Blum, H.E., Penin, F., Moradpour, D., 2002. An amino-terminal amphipathic alpha-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J. Biol. Chem. 277 (10), 8130–8139.
Bressanelli, S., Tomei, L., Roussel, A., Incitti, I., Vitale, R.L., Mathieu, M., De Francesco, R., Rey, F.A., 1999. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. Proc. Natl. Acad. Sci. 96 (23), 13034–13039.
Briggs, M.W., Sacks, D.B., 2003. IQGAP proteins are integral components of cytoskeletal regulation. EMBO Rep. 4 (6), 571–574.
Bruschke, C., Hulst, M.M., Moormann, R., Van Rijn, P., Van Oirschot, J., 1997. Glycoprotein Erns of pestiviruses induces apoptosis in lymphocytes of several species. J. Virol. 71 (9), 6692–6696.
Chen, W., Yan, W., Du, Q., Fei, L., Liu, M., Ni, Z., Sheng, Z., Zheng, Z., 2004. RNA interference targeting VP1 inhibits foot-and-mouth disease virus replication in BHK-21 cells and suckling mice. J. Virol. 78 (13), 6900–6907.
Chen, Y., Xiao, J., Xiao, J., Sheng, C., Wang, J., Jia, L., Zhi, Y., Li, G., Chen, J., Xiao, M., 2012. Classical swine fever virus NS5A regulates viral RNA replication through binding to NS5B and 3′ UTR. Virology 432 (2), 376–388.
Chen, Z., Rijnbrand, R., Jangra, R.K., Devaraj, S.G., Qu, L., Ma, Y., Lemon, S.M., Li, K., 2007. Ubiquitination and proteasomal degradation of interferon regulatory factor-3 induced by Npro from a cytopathic bovine viral diarrhea virus. Virology 366 (2), 277–292.
Chiu, M.-W., Shih, H.-M., Yang, T.-H., Yang, Y.-L., 2007. The type 2 dengue virus envelope protein interacts with small ubiquitin-like modifier-1 (SUMO-1) conjugating enzyme 9 (Ubc9). J. Biomed. Sci. 14 (3), 429–444.
Choi, K.H., Groarke, J.M., Young, D.C., Kuhn, R.J., Smith, J.L., Pevear, D.C., Rossmann, M.G., 2004. The structure of the RNA-dependent RNA polymerase from bovine

viral diarrhea virus establishes the role of GTP in de novo initiation. Proc. Natl. Acad. Sci. U.S.A. 101 (13), 4425–4430.
David, M., Knipe, P.H., 2013a. Flaviviridae: Hepatitis C viruses: features of the non-structural proteins. In: Fields Virology, sixth ed. Lippincott Williams and Wilkins, pp. 733.
David, M., Knipe, P.H., 2013b. Flaviviridae: pestiviruses: assembly and release of virus particles. In: Fields Virology, sixth ed. Lippincott Williams and Wilkins, pp. 738–739.
Dong, X.N., Chen, Y.H., 2007. Marker vaccine strategies and candidate CSFV marker vaccines. Vaccine 25 (2), 205–230.
Dreux, M., Gastaminza, P., Wieland, S.F., Chisari, F.V., 2009. The autophagy machinery is required to initiate hepatitis C virus replication. Proc. Natl. Acad. Sci. 106 (33), 14046–14051.
Edwards, S., Fukusho, A., Lefevre, P.-C., Lipowski, A., Pejsak, Z., Roehe, P., Westergaard, J., 2000. Classical swine fever: the global situation. Vet. Microbiol. 73 (2), 103–119.
El Omari, K., Iourin, O., Harlos, K., Grimes, J.M., Stuart, D.I., 2013. Structure of a pestivirus envelope glycoprotein E2 clarifies its role in cell entry. Cell Rep. 3 (1), 30–35.
Elbers, K., Tautz, N., Becher, P., Stoll, D., Rümenapf, T., Thiel, H.-J., 1996. Processing in the pestivirus E2-NS2 region: identification of proteins p7 and E2p7. J. Virol. 70 (6), 4131–4135.
Fan, Y.-f., Zhao, Q.-z., Zhao, Y., Zou, X.-q., Zhang, Z.-q., Wang, Q., Ning, Y.-b., 2009. The study of recovered infectious classical swine fever virus from full-length cDNA clone. China Anim. Husb. Vet. Med. 6, 019.
Fan, Z.-C., Bird, R.C., 2008. Generation and characterization of an Npro-disrupted marker bovine viral diarrhea virus derived from a BAC cDNA. J. Virol. Methods 151 (2), 257–263.
Fernández-Sainz, I., Largo, E., Gladue, D., Fletcher, P., O'Donnell, V., Holinka, L., Carey, L., Lu, X., Nieva, J., Borca, M., 2014. Effect of specific amino acid substitutions in the putative fusion peptide of structural glycoprotein E2 on classical swine fever virus replication. Virology 456, 121–130.
Fernandez-Sainz, I., Gladue, D., Holinka, L., O'Donnell, V., Gudmundsdottir, I., Prarat, M., Patch, J., Golde, W., Lu, Z., Zhu, J., 2010. Mutations in classical swine fever virus NS4B affect virulence in swine. J. Virol. 84 (3), 1536–1549.
Fernandez-Sainz, I., Holinka, L.G., Gavrilov, B.K., Prarat, M.V., Gladue, D., Lu, Z., Jia, W., Risatti, G.R., Borca, M.V., 2009. Alteration of the N-linked glycosylation condition in E1 glycoprotein of classical swine fever virus strain Brescia alters virulence in swine. Virology 386 (1), 210–216.
Fiebach, A.R., Guzylack-Piriou, L., Python, S., Summerfield, A., Ruggli, N., 2011. Classical swine fever virus Npro limits type I interferon induction in plasmacytoid dendritic cells by interacting with interferon regulatory factor 7. J. Virol. 85 (16), 8002–8011.
Fletcher, S.P., Ali, I.K., Kaminski, A., Digard, P., Jackson, R.J., 2002. The influence of viral coding sequences on pestivirus IRES activity reveals further parallels with translation initiation in prokaryotes. RNA 8 (12), 1558–1571.
Fletcher, S.P., Jackson, R.J., 2002. Pestivirus internal ribosome entry site (IRES) structure and function: elements in the 5′ untranslated region important for IRES function. J. Virol. 76 (10), 5024–5033.
Friebe, P., Lohmann, V., Krieger, N., Bartenschlager, R., 2001. Sequences in the 5′ nontranslated region of hepatitis C virus required for RNA replication. J. Virol. 75 (24), 12047–12057.
Gavrilov, B.K., Rogers, K., Fernandez-Sainz, I.J., Holinka, L.G., Borca, M.V., Risatti, G.R., 2011. Effects of glycosylation on antigenicity and immunogenicity of classical swine fever virus envelope proteins. Virology 420 (2), 135–145.
Ge, Q., McManus, M.T., Nguyen, T., Shen, C.-H., Sharp, P.A., Eisen, H.N., Chen, J., 2003. RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. Proc. Natl. Acad. Sci. 100 (5), 2718–2723.
Gil, L.H., Ansari, I.H., Vassilev, V., Liang, D., Lai, V.C., Zhong, W., Hong, Z., Dubovi, E.J., Donis, R.O., 2006. The amino-terminal domain of bovine viral diarrhea virus Npro protein is necessary for alpha/beta interferon antagonism. J. Virol. 80 (2), 900–911.
Gladue, D., Holinka, L., Fernandez-Sainz, I., Prarat, M., O'donell, V., Vepkhvadze, N., Lu, Z., Rogers, K., Risatti, G., Borca, M., 2010. Effects of the interactions of classical swine fever virus Core protein with proteins of the SUMOylation pathway on virulence in swine. Virology 407 (1), 129–136.
Gladue, D., Holinka, L., Fernandez-Sainz, I., Prarat, M., O'Donnell, V., Vepkhvadze, N., Lu, Z., Risatti, G., Borca, M., 2011a. Interaction between core protein of classical swine fever virus with cellular IQGAP1 protein appears essential for virulence in swine. Virology 412 (1), 68–74.
Gladue, D., O'Donnell, V., Fernandez-Sainz, I., Fletcher, P., Baker-Branstetter, R., Holinka, L., Sanford, B., Carlson, J., Lu, Z., Borca, M., 2014a. Interaction of structural core protein of classical swine fever virus with endoplasmic reticulum-associated degradation pathway protein OS9. Virology 460, 173–179.
Gladue, D.P., Baker-Bransetter, R., Holinka, L.G., Fernandez-Sainz, I.J., O'Donnell, V., Fletcher, P., Lu, Z., Borca, M.V., 2014b. Interaction of CSFV E2 protein with swine host factors as detected by yeast two-hybrid system. PLoS One 9 (1), e85324.
Gladue, D.P., Gavrilov, B.K., Holinka, L.G., Fernandez-Sainz, I.J., Vepkhvadze, N., Rogers, K., O'Donnell, V., Risatti, G.R., Borca, M.V., 2011b. Identification of an NTPase motif in classical swine fever virus NS4B protein. Virology 411 (1), 41–49.
Gladue, D.P., Holinka, L.G., Largo, E., Fernandez Sainz, I., Carrillo, C., O'Donnell, V., Baker-Branstetter, R., Lu, Z., Ambroggio, X., Risatti, G.R., Nieva, J.L., Borca, M.V., 2012. Classical swine fever virus p7 protein is a viroporin involved in virulence in swine. J. Virol. 86 (12), 6778–6791.
Gong, Y., Trowbridge, R., Macnaughton, T.B., Westaway, E.G., Shannon, A.D., Gowans, E.J., 1996. Characterization of RNA synthesis during a one-step growth curve and of the replication mechanism of bovine viral diarrhoea virus. J. Gen. Virol. 77 (11), 2729–2736.
Gorbalenya, A.E., Koonin, E.V., 1989a. Viral proteins containing the purine NTP-binding sequence pattern. Nucleic Acids Res. 17 (21), 8413–8438.
Gottipati, K., Ruggli, N., Gerber, M., Tratschin, J.-D., Benning, M., Bellamy, H., Choi, K.H., 2013. The structure of classical swine fever virus Npro: a novel cysteine autoprotease and zinc-binding protein involved in subversion of Type I interferon induction. PLoS Pathog. 9 (10), e1003704.
Grassmann, C.W., Isken, O., Tautz, N., Behrens, S.-E., 2001. Genetic analysis of the pestivirus nonstructural coding region: defects in the NS5A unit can be complemented intrans. J. Virol. 75 (17), 7791–7802.
Guo, H.-C., Sun, S.-Q., Sun, D.-H., Wei, Y.-Q., Xu, J., Huang, M., Liu, X.-T., Liu, Z.-X., Luo, J.-X., Yin, H., Liu, D.X., 2013a. Viroporin activity and membrane topology of classic swine fever virus p7 protein. Int. J. Biochem. Cell Biol. 45 (7), 1186–1194.
Guo, K.-K., Tang, Q.-H., Ning, P.-B., Li, H.-L., Liu, W., Lv, Q.-Z., Liang, W.-L., Lin, Z., Zhang, C.-C., Zhang, Y.-M., 2013b. Pilot study on degradation of classical swine fever virus nonstructural 2 protein in cells. J. Anim. Vet. Adv. 12 (2), 234–241.
Haegeman, A., Vrancken, R., Neyts, J., Koenen, F., 2013. Intra-host variation structure of classical swine fever virus NS5B in relation to antiviral therapy. Antivir. Res. 98 (2), 266–272.
Han, Y., Chen, Y.-S., Liu, Z., Bodyak, N., Rigor, D., Bisping, E., Pu, W.T., Kang, P.M., 2006. Overexpression of HAX-1 protects cardiac myocytes from apoptosis through caspase-9 inhibition. Circ. Res. 99 (4), 415–423.
Harada, T., Tautz, N., Thiel, H.-J., 2000. E2-p7 region of the bovine viral diarrhea virus polyprotein: processing and functional studies. J. Virol. 74 (20), 9498–9506.
Haseloff, J., Gerlach, W.L., 1988. Simple RNA enzymes with new and highly specific endoribonuclease activities. Nature 334 (6183), 585–591.
Hausmann, Y., Roman-Sosa, G., Thiel, H.-J., Rümenapf, T., 2004. Classical swine fever virus glycoprotein Erns is an endoribonuclease with an unusual base specificity. J. Virol. 78 (10), 5507–5512.
He, D.N., Zhang, X.M., Liu, K., Pang, R., Zhao, J., Zhou, B., Chen, P.Y., 2014. In vitro inhibition of the replication of classical swine fever virus by porcine Mx1 protein. Antivir. Res. 104, 128–135.
He, F., Ling, L., Liao, Y., Li, S., Han, W., Zhao, B., Sun, Y., Qiu, H.-J., 2013. Beta-actin interacts with the E2 protein and is involved in the early replication of classical swine fever virus. Virus Res.
He, Y.-x., Hua, R.-h., Zhou, Y.-j., Qiu, H.-j., Tong, G.-z., 2007. Interference of porcine reproductive and respiratory syndrome virus replication on MARC-145 cells using DNA-based short interfering RNAs. Antivir. Res. 74 (2), 83–91.
Heimann, M., Sosa, G.R., Martoglio, B., Thiel, H.-J., Rümenapf, T., 2006. Core protein of pestiviruses is processed at the C terminus by signal peptide peptidase. J. Virol. 80 (4), 1915–1921.
Hellen, C.U., Sarnow, P., 2001. Internal ribosome entry sites in eukaryotic mRNA molecules. Genes Dev. 15 (13), 1593–1612.
Hsu, W.-L., Chen, C.-L., Huang, S.-W., Wu, C.-C., Chen, I.-H., Nadar, M., Su, Y.-P., Tsai, C.-H., 2014. The untranslated regions of classic swine fever virus rna trigger apoptosis. PLoS One 9 (2), e88863.
Huang, J.-h., Li, Y.-f., He, F., Li D D., Sun, Y., Han, W., Qiu, H.-j., 2013. Rapid recovery of classical swine fever virus directly from cloned cDNA. J. Integr. Agric. 12 (5), 877–883.
Huang, Y.-L., Deng, M.-C., Wang, F.-I., Huang, C.-C., Chang, C.-Y., 2014. The challenges of classical swine fever control: modified live and E2 subunit vaccines. Virus Res. 179, 1–11.
Huh, Y.H., Kim, Y.E., Kim, E.T., Park, J.J., Song, M.J., Zhu, H., Hayward, G.S., Ahn, J.-H., 2008. Binding STAT2 by the acidic domain of human cytomegalovirus IE1 promotes viral growth and is negatively regulated by SUMO. J. Virol. 82 (21), 10444–10454.
Hulst, M., Moormann, R., 1997. Inhibition of pestivirus infection in cell culture by envelope proteins Erns and E2 of classical swine fever virus: Erns and E2 interact with different receptors. J. Gen. Virol. 78 (11), 2779–2787.
Hulst, M., Panoto, F., Hoekman, A., Van Gennip, H., Moormann, R., 1998. Inactivation of the RNase activity of glycoprotein Erns of classical swine fever virus results in a cytopathogenic virus. J. Virol. 72 (1), 151–157.
Irie, M., Ohgi, K., 2001. Ribonuclease T2. Method Enzymol. 341, 42.
Iwasaki, A., Medzhitov, R., 2010. Regulation of adaptive immunity by the innate immune system. Science 327 (5963), 291–295.
Ji, W., Niu, D.D., Si, H.L., Ding, N.Z., He, C.Q., 2014. Vaccination influences the evolution of classical swine fever virus. Infect. Genet. Evol. 25, 69–77.
Johns, H.L., Bensaude, E., La Rocca, S.A., Seago, J., Charleston, B., Steinbach, F., Drew, T.W., Crooke, H., Everett, H., 2010a. Classical swine fever virus infection protects aortic endothelial cells from pIpC-mediated apoptosis. J. Gen. Virol. 91 (4), 1038–1046.
Johns, H.L., Doceul, V., Everett, H., Crooke, H., Charleston, B., Seago, J., 2010b. The classical swine fever virus N-terminal protease Npro binds to cellular HAX-1. J. Gen. Virol. 91 (11), 2677–2686.
Koprowski, H., James, T.R., Cox, H.R., 1946. Propagation of hog cholera virus in rabbits. Proc. Soc. Exp. Biol. Med. 63, 178–183 (Society for Experimental Biology and Medicine (New York, NY)).
Krey, T., Bontems, F., Vonrhein, C., Vaney, M.-C., Bricogne, G., Rümenapf, T., Rey, Félix A., 2012. Crystal structure of the pestivirus envelope glycoprotein Erns and mechanistic analysis of its ribonuclease activity. Structure 20 (5), 862–873.
Kudoh, A., Fujita, M., Kiyono, T., Kuzushima, K., Sugaya, Y., Izuta, S., Nishiyama, Y., Tsurumi, T., 2003. Reactivation of lytic replication from B cells latently infected

with Epstein-Barr virus occurs with high S-phase cyclin-dependent kinase activity while inhibiting cellular DNA replication. J. Virol. 77 (2), 851–861.
Kyei, G.B., Dinkins, C., Davis, A.S., Roberts, E., Singh, S.B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., 2009. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. J. Cell Biol. 186 (2), 255–268.
Lackner, T., Thiel, H.-J., Tautz, N., 2006. Dissection of a viral autoprotease elucidates a function of a cellular chaperone in proteolysis. Proc. Natl. Acad. Sci. U.S.A. 103 (5), 1510–1515.
Lai, C.-K., Jeng, K.-S., Machida, K., Lai, M.M., 2008. Association of hepatitis C virus replication complexes with microtubules and actin filaments is dependent on the interaction of NS3 and NS5A. J. Virol. 82 (17), 8838–8848.
Lai, V.C., Kao, C.C., Ferrari, E., Park, J., Uss, A.S., Wright-Minogue, J., Hong, Z., Lau, J.Y., 1999. Mutational analysis of bovine viral diarrhea virus RNA-dependent RNA polymerase. J. Virol. 73 (12), 10129–10136.
Lamp, B., Riedel, C., Wentz, E., Tortorici, M.-A., Rümenapf, T., 2013. Autocatalytic cleavage within classical swine fever virus NS3 leads to a functional separation of protease and helicase. J. Virol. 87 (21), 11872–11883.
Langedijk, J., Van Veelen, P., Schaaper, W., De Ru, A., Meloen, R., Hulst, M., 2002. A structural model of pestivirus Erns based on disulfide bond connectivity and homology modeling reveals an extremely rare vicinal disulfide. J. Virol. 76 (20), 10383–10392.
Langedijk, J.P., 2002. Translocation activity of C-terminal domain of pestivirus Erns and ribotoxin L3 loop. J. Biol. Chem. 277 (7), 5308–5314.
Lecot, S., Belouzard, S., Dubuisson, J., Rouillé, Y., 2005. Bovine viral diarrhea virus entry is dependent on clathrin-mediated endocytosis. J. Virol. 79 (16), 10826–10829.
Lee, Y.-R., Lei, H.-Y., Liu, M.-T., Wang, J.-R., Chen, S.-H., Jiang-Shieh, Y.-F., Lin, Y.-S., Yeh, T.-M., Liu, C.-C., Liu, H.-S., 2008. Autophagic machinery activated by dengue virus enhances virus replication. Virology 374 (2), 240–248.
Leifer, I., Hoeper, D., Blome, S., Beer, M., Ruggli, N., 2011. Clustering of classical swine fever virus isolates by codon pair bias. BMC Res. Notes 4 (1), 521.
Leifer, I., Ruggli, N., Blome, S., 2013. Approaches to define the viral genetic basis of classical swine fever virus virulence. Virology 438 (2), 51–55.
Li, C., Huang, J., Li, Y., He, F., Li, D., Sun, Y., Han, W., Li, S., Qiu, H.-J., 2013a. Efficient and stable rescue of classical swine fever virus from cloned cDNA using an RNA polymerase II system. Arch. Virol. 158 (4), 901–907.
Li, C., Li, Y., Shen, L., Huang, J., Sun, Y., Luo, Y., Zhao, B., Wang, C., Yuan, J., Qiu, H.-J., 2014a. The role of noncoding regions of classical swine fever virus C-strain in its adaptation to the rabbit. Virus Res. 183, 117–122.
Li, W., Zhang, Y., Kao, C.C., 2014b. The classic swine fever virus (CSFV) core protein can enhance de novo-initiated RNA synthesis by the CSFV polymerase NS5B. Virus Genes 49 (1), 106–115.
Li, Y., Shen, L., Li, C., Huang, J., Zhao, B., Sun, Y., Li, S., Luo, Y., Qiu, H.-J., 2014c. Visualization of the Npro protein in living cells using biarsenically labeling tetracysteine-tagged classical swine fever virus. Virus Res.
Li, Y., Shen, L., Sun, Y., Yuan, J., Huang, J., Li, C., Li, S., Luo, Y., Qiu, H.-J., 2013b. Simplified serum neutralization test based on enhanced green fluorescent protein-tagged classical swine fever virus. J. Clin. Microbiol. 51 (8), 2710–2712.
Li, Y., Wang, J., Kanai, R., Modis, Y., 2013c. Crystal structure of glycoprotein E2 from bovine viral diarrhea virus. Proc. Natl. Acad. Sci. 110 (17), 6805–6810.
Liang, M.-H., Geisbert, T., Yao, Y., Hinrichs, S.H., Giam, C.-Z., 2002. Human T-lymphotropic virus type 1 oncoprotein tax promotes S-phase entry but blocks mitosis. J. Virol. 76 (8), 4022–4033.
Liess, B., 1988. Classical Swine Fever and Related Viral Infections. Springer, pp. 165–180.
Lin, T.C., Shieh, C.M., Su, J.F., Chen, C., 1972. Pathogenicity of the hog cholera live vaccine viruses by reverse passages through SPF pigs. Exp. Rep. Taiwan Prov. Res. Inst. Anim. Hlth. 9, 1–5.
Lin, T.C., Shieh, C.M., Su, J.F., 1974. Virus multiplication in pigs inoculated with lapinized hog cholera live vaccine. Zhonghua Minguo wei sheng wu xue za zhi 7 (1), 13–19.
Lin, T.T.C., Lee, R.C.T., Joint Commission on Rural Reconstruction, 1981. An Overall Report on the Development of a Highly Safe and Potent Lapinized Hog Cholera Virus Strain for Hog Cholera Control in Taiwan. National Science Council.
Lin, Z., Liang, W., Kang, K., Li, H., Cao, Z., Zhang, Y., 2014. Classical swine fever virus (CSFV) and p7 protein induce secretion of IL-1β in Macrophages. J. Gen. Virol. 95 (Pt 12), 2693–2699.
Liu, J.-J., Wong, M.-L., Chang, T.-J., 1998. The recombinant nucleocapsid protein of classical swine fever virus can act as a transcriptional regulator. Virus Res. 53 (1), 75–80.
Lohmann, V., Körner, F., Herian, U., Bartenschlager, R., 1997. Biochemical properties of hepatitis C virus NS5B RNA-dependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity. J. Virol. 71 (11), 8416–8428.
LunaisM, A., 1972. Classical swine fever: properties of a clone (strain"Thiverval") isolated from a cellular culture at a low temperature: application in vaccination. Rev. Med. Vet. (Toulouse) 123 (12), 1537–1554.
Luo, Y., Li, S., Sun, Y., Qiu, H.-J., 2014. Classical swine fever in China: a minireview. Vet. Microbiol. 172 (1–2), 1–6.
Mätzener, P., Magkouras, I., Rümenapf, T., Peterhans, E., Schweizer, M., 2009. The viral RNase Erns prevents IFN type-I triggering by pestiviral single- and double-stranded RNAs. Virus Res. 140 (1–2), 15–23.
Magkouras, I., Mätzener, P., Rümenapf, T., Peterhans, E., Schweizer, M., 2008. RNase-dependent inhibition of extracellular, but not intracellular, dsRNA-induced interferon synthesis by Erns of pestiviruses. J. Gen. Virol. 89 (10), 2501–2506.
Mayer, D., Hofmann, M.A., Tratschin, J.-D., 2004. Attenuation of classical swine fever virus by deletion of the viral Npro gene. Vaccine 22 (3), 317–328.
Meyers, G., Saalmüller, A., Büttner, M., 1999. Mutations abrogating the RNase activity in glycoprotein Erns of the pestivirus classical swine fever virus lead to virus attenuation. J. Virol. 73 (12), 10224–10235.
Meyers, G., Thiel, H.-J., Rümenapf, T., 1996. Classical swine fever virus: recovery of infectious viruses from cDNA constructs and generation of recombinant cytopathogenic defective interfering particles. J. Virol. 70 (3), 1588–1595.
Moormann, R., Van Gennip, H., Miedema, G., Hulst, M., Van Rijn, P., 1996. Infectious RNA transcribed from an engineered full-length cDNA template of the genome of a pestivirus. J. Virol. 70 (2), 763–770.
Moser, C., Stettler, P., Tratschin, J.-D., Hofmann, M.A., 1999. Cytopathogenic and noncytopathogenic RNA replicons of classical swine fever virus. J. Virol. 73 (9), 7787–7794.
Moulin, H.R., Seuberlich, T., Bauhofer, O., Bennett, L.C., Tratschin, J.-D., Hofmann, M.A., Ruggli, N., 2007. Nonstructural proteins NS2–3 and NS4A of classical swine fever virus: essential features for infectious particle formation. Virology 365 (2), 376–389.
Nishimura, Y., Sato, U., Hanaki, T., Nobuto, K., 1964. Studies on the tissue culture of hog cholera virus. II. Neutralization test by means of the influence of hog cholera virus infection on Newcastle disease virus infection (HEIC method). Nihon juigaku zasshi 26 (3), 133–140.
OIE, 2014. List of Countries by Classical Swine Fever Disease Situation.
Olah, P., 1985. Study of the Suvac Strain of Lapinized Swine Fever Virus.
Orvedahl, A., Alexander, D., Tallóczy, Z., Sun, Q., Wei, Y., Zhang, W., Burns, D., Leib, D.A., Levine, B., 2007. HSV-1 ICP34, 5 confers neurovirulence by targeting the Beclin 1 autophagy protein. Cell Host Microbe 1 (1), 23–35.
Palacios, S., Perez, L.H., Welsch, S., Schleich, S., Chmielarska, K., Melchior, F., Locker, J.K., 2005. Quantitative SUMO-1 modification of a vaccinia virus protein is required for its specific localization and prevents its self-association. Mol. Biol. Cell 16 (6), 2822–2835.
Park, G.-S., Lim, S.-I., Hong, S.-H., Song, J.-Y., 2012. Establishment and characterization of an infectious cDNA clone of a classical swine fever virus LOM strain. J. Vet. Sci. 13 (1), 81–91.
Pei, J., Zhao, M., Ye, Z., Gou, H., Wang, J., Yi, L., Dong, X., Liu, W., Luo, Y., Liao, M., 2013. Autophagy enhances the replication of classical swine fever virus in vitro. Autophagy 10 (1), 93–110.
Pejsak, Z.L.A., Markowska, I., Dzierzawski, A., Mokrzycka, A., 1991. Immunogenicity and safety of the new attenuated Cellpest vaccine against swine fever. Med. Weter. 47 (10), 461–463.
Porntrakulpipat, S., Supankong, S., Chatchawanchonteera, A., Pakdee, P., 2010. RNA interference targeting nucleocapsid protein (C) inhibits classical swine fever virus replication in SK-6 cells. Vet. Microbiol. 142 (1), 41–44.
Postel, A., Schmeiser, S., Bernau, J., Meindl-Boehmer, A., Pridotkas, G., Dirbakova, Z., Mojzis, M., Becher, P., 2012. Improved strategy for phylogenetic analysis of classical swine fever virus based on full-length E2 encoding sequences. Vet. Res. 43, 50.
Rümenapf, T., Stark, R., Heimann, M., Thiel, H.-J., 1998. N-terminal protease of pestiviruses: identification of putative catalytic residues by site-directed mutagenesis. J. Virol. 72 (3), 2544–2547.
Rümenapf, T., Unger, G., Strauss, J.H., Thiel, H.-J., 1993. Processing of the envelope glycoproteins of pestiviruses. J. Virol. 67 (6), 3288–3294.
Rawlings, N.D., Waller, M., Barrett, A.J., Bateman, A., 2014. MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res. 42 (D1), D503–D509.
Rice, L., 2001. Flaviviridae: the viruses and their replication. In: Fields Virology. Lippincott Williams and Wilkins, pp. 991–1041.
Riedel, C., Lamp, B., Heimann, M., Rümenapf, T., 2010. Characterization of essential domains and plasticity of the classical swine fever virus core protein. J. Virol. 84 (21), 11523–11531.
Risatti, G., Borca, M., Kutish, G., Lu, Z., Holinka, L., French, R., Tulman, E., Rock, D., 2005b. The E2 glycoprotein of classical swine fever virus is a virulence determinant in swine. J. Virol. 79 (6), 3787–3796.
Risatti, G., Holinka, L., Carrillo, C., Kutish, G., Lu, Z., Tulman, E., Sainz, I.F., Borca, M., 2006. Identification of a novel virulence determinant within the E2 structural glycoprotein of classical swine fever virus. Virology 355 (1), 94–101.
Risatti, G., Holinka, L., Fernandez Sainz, I., Carrillo, C., Kutish, G., Lu, Z., Zhu, J., Rock, D., Borca, M., 2007b. Mutations in the carboxyl terminal region of E2 glycoprotein of classical swine fever virus are responsible for viral attenuation in swine. Virology 364 (2), 371–382.
Risatti, G., Holinka, L., Lu, Z., Kutish, G., Tulman, E., French, R., Sur, J., Rock, D., Borca, M., 2005a. Mutation of E1 glycoprotein of classical swine fever virus affects viral virulence in swine. Virology 343 (1), 116–127.
Risatti, G., Holinka, L., Sainz, I.F., Carrillo, C., Lu, Z., Borca, M., 2007a. N-linked glycosylation status of classical swine fever virus strain Brescia E2 glycoprotein influences virulence in swine. J. Virol. 81 (2), 924–933.
Ruggli, N., Bird, B.H., Liu, L., Bauhofer, O., Tratschin, J.-D., Hofmann, M.A., 2005. Npro of classical swine fever virus is an antagonist of double-stranded RNA-mediated apoptosis and IFN-α/β induction. Virology 340 (2), 265–276.
Ruggli, N., Tratschin, J.-D., Schweizer, M., McCullough, K.C., Hofmann, M.A., Summerfield, A., 2003. Classical swine fever virus interferes with cellular antiviral defense: evidence for a novel function of Npro. J. Virol. 77 (13), 7645–7654.
Sainz, I.F., Holinka, L.G., Lu, Z., Risatti, G.R., Borca, M.V., 2008. Removal of a N-linked glycosylation site of classical swine fever virus strain Brescia Erns glycoprotein affects virulence in swine. Virology 370 (1), 122–129.

Sasahara, J., Kumagai, T., Shimizu, Y., Furuuchi, S., 1969. Field experiments of hog cholera live vaccine prepared in guinea-pig kidney cell culture. Natl. Inst. Anim. Health Q. 9 (2), 83.
Seago, J., Hilton, L., Reid, E., Doceul, V., Jeyatheesan, J., Moganeradj, K., McCauley, J., Charleston, B., Goodbourn, S., 2007. The Npro product of classical swine fever virus and bovine viral diarrhea virus uses a conserved mechanism to target interferon regulatory factor-3. J. Gen. Virol. 88 (11), 3002–3006.
Sharp, T.V., Wang, H.-W., Koumi, A., Hollyman, D., Endo, Y., Ye, H., Du, M.-Q., Boshoff, C., 2002. K15 protein of Kaposi's sarcoma-associated herpesvirus is latently expressed and binds to HAX-1, a protein with antiapoptotic function. J. Virol. 76 (2), 802–816.
Shen, L., Li, Y., Chen, J., Li, C., Huang, J., Luo, Y., Sun, Y., Li, S., Qiu, H.-J., 2014. Generation of a recombinant classical swine fever virus stably expressing the firefly luciferase gene for quantitative antiviral assay. Antivir. Res. 109, 15–21.
Sheng, C., Chen, Y., Xiao, J., Xiao, J., Wang, J., Li, G., Chen, J., Xiao, M., 2012. Classical swine fever virus NS5A protein interacts with 3′-untranslated region and regulates viral RNA synthesis. Virus Res. 163 (2), 636–643.
Sheng, C., Xiao, M., Geng, X., Liu, J., Wang, Y., Gu, F., 2007. Characterization of interaction of classical swine fever virus NS3 helicase with 3′ untranslated region. Virus Res. 129 (1), 43–53.
Sheng, C., Zhu, Z., Yu, J., Wan, L., Wang, Y., Chen, J., Gu, F., Xiao, M., 2010. Characterization of NS3, NS5A and NS5B of classical swine fever virus through mutation and complementation analysis. Vet. Microbiol. 140 (1), 72–80.
Shimizu, Y., Furuuchi, S., Kumagai, T., Sasahara, J., 1970. A mutant of hog cholera virus inducing interference in swine testicle cell cultures. Am. J. Vet. Res. 31, 1787–1794.
Shirai, C., Mizuta, K., 2008. SUMO mediates interaction of Ebp2p, the yeast homolog of Epstein-Barr virus nuclear antigen 1-binding protein 2, with a RING finger protein Ris1p. Biosci. Biotechnol. Biochem., 0806060934.
Sieczkarski, S.B., Whittaker, G.R., 2002. Dissecting virus entry via endocytosis. J. Gen. Virol. 83 (7), 1535–1545.
Szymanski, M.R., Fiebach, A.R., Tratschin, J.-D., Gut, M., Ramanujam, V., Gottipati, K., Patel, P., Ye, M., Ruggli, N., Choi, K.H., 2009. Zinc binding in pestivirus Npro is required for interferon regulatory Factor 3 interaction and degradation. J. Mol. Biol. 391 (2), 438–449.
Tamura, T., Nagashima, N., Ruggli, N., Summerfield, A., Kida, H., Sakoda, Y., 2014. Npro of classical swine fever virus contributes to pathogenicity in pigs by preventing type I interferon induction at local replication sites. Vet. Res. 45, 47.
Tamura, T., Sakoda, Y., Yoshino, F., Nomura, T., Yamamoto, N., Sato, Y., Okamatsu, M., Ruggli, N., Kida, H., 2012. Selection of classical swine fever virus with enhanced pathogenicity reveals synergistic virulence determinants in E2 and NS4B. J. Virol. 86 (16), 8602–8613.
Tang, Q., Guo, K., Kang, K., Zhang, Y., He, L., Wang, J., 2011. Classical swine fever virus NS2 protein promotes interleukin-8 expression and inhibits MG132-induced apoptosis. Virus Genes 42 (3), 355–362.
Tang, Q., Zhang, Y., Fan, L., Tong, G., He, L., Dai, C., 2010. Classic swine fever virus NS2 protein leads to the induction of cell cycle arrest at S-phase and endoplasmic reticulum stress. Virol. J. 7 (4).
Tarradas, J., de la Torre, M.E., Rosell, R., Perez, L.J., Pujols, J., Muñoz, M., Muñoz, I., Muñoz, S., Abad, X., Domingo, M., 2014. The impact of CSFV on the immune response to control infection. Virus Res. 185, 82–91.
Tautz, N., Elbers, K., Stoll, D., Meyers, G., Thiel, H., 1997. Serine protease of pestiviruses: determination of cleavage sites. J. Virol. 71 (7), 5415–5422.
Tautz, N., Kaiser, A., Thiel, H.-J., 2000. NS3 serine protease of bovine viral diarrhea virus: characterization of active site residues, NS4A cofactor domain, and protease–cofactor interactions. Virology 273 (2), 351–363.
Tc, Z., 1980. Progress of classical swine fever virus and the prevention of the disease (partII). Chin J Vet sci Technol 5, 30–39.
Terpstra, C., 1992. Swine kidney cell culture, and its use in vaccine production. Google Patents.
Tews, B.A., Schürmann, E.-M., Meyers, G., 2009. Mutation of cysteine 171 of pestivirus Erns RNase prevents homodimer formation and leads to attenuation of classical swine fever virus. J. Virol. 83 (10), 4823–4834.
Thiel, H., Stark, R., Weiland, E., Rümenapf, T., Meyers, G., 1991. Hog cholera virus: molecular composition of virions from a pestivirus. J. Virol. 65 (9), 4705–4712.
Tratschin, J.-D., Moser, C., Ruggli, N., Hofmann, M.A., 1998. Classical swine fever virus leader proteinase Npro is not required for viral replication in cell culture. J. Virol. 72 (9), 7681–7684.
Van Gennip, H., Van Rijn, P., Widjojoatmodjo, M., Moormann, R., 1999. Recovery of infectious classical swine fever virus (CSFV) from full-length genomic cDNA clones by a swine kidney cell line expressing bacteriophage T7 RNA polymerase. J. Virol. Methods 78 (1), 117–128.
Van Gennip, H.G., Vlot, A.C., Hulst, M.M., De Smit, A.J., Moormann, R.J., 2004. Determinants of virulence of classical swine fever virus strain Brescia. J. Virol. 78 (16), 8812–8823.
van Gennip, H.G.P., van Rijn, P.A., Widjojoatmodjo, M.N., de Smit, A.J., Moormann, R.J.M., 2000. Chimeric classical swine fever viruses containing envelope protein ERNS or E2 of bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a distinguishable antibody response. Vaccine 19 (4–5), 447–459.
Van Oirschot, J., 2003. Vaccinology of classical swine fever: from lab to field. Vet. Microbiol. 96 (4), 367–384.
Van Rijn, P., Miedema, G., Wensvoort, G., Van Gennip, H., Moormann, R., 1994. Antigenic structure of envelope glycoprotein E1 of hog cholera virus. J. Virol. 68 (6), 3934–3942.
Van Rijn, P., Van Gennip, H., De Meijer, E., Moormann, R., 1993. Epitope mapping of envelope glycoprotein E1 of hog cholera virus strain Brescia. J. Gen. Virol. 74, 2053–2060.
Voigt, H., Wienhold, D., Marquardt, C., Muschko, K., Pfaff, E., Buettner, M., 2007. Immunity against NS3 protein of classical swine fever virus does not protect against lethal challenge infection. Viral Immunol. 20 (3), 487–494.
Vrancken, R., Haegeman, A., Dewulf, J., Paeshuyse, J., Puerstinger, G., Tignon, M., Le Potier, M.F., Neyts, J., Koenen, F., 2009a. The reduction of CSFV transmission to untreated pigs by the pestivirus inhibitor BPIP: a proof of concept. Vet. Microbiol. 139 (3–4), 365–368.
Vrancken, R., Paeshuyse, J., Haegeman, A., Puerstinger, G., Froeyen, M., Herdewijn, P., Kerkhofs, P., Neyts, J., Koenen, F., 2008. Imidazo[4,5-c]pyridines inhibit the in vitro replication of the classical swine fever virus and target the viral polymerase. Antivir. Res. 77 (2), 114–119.
Walker, J.E., Saraste, M., Runswick, M.J., Gay, N.J., 1982. Distantly related sequences in the alpha-and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. 1 (8), 945.
Wang, P., Wang, Y., Zhao, Y., Zhu, Z., Yu, J., Wan, L., Chen, J., Xiao, M., 2010. Classical swine fever virus NS3 enhances RNA-dependent RNA polymerase activity by binding to NS5B. Virus Res. 148 (1), 17–23.
Wang, Y., Xiao, M., Chen, J., Zhang, W., Luo, J., Bao, K., Nie, M., Chen, J., Li, B., 2007. Mutational analysis of the GDD sequence motif of classical swine fever virus RNA-dependent RNA polymerases. Virus Genes 34 (1), 63–65.
Wang, Y., Zhu, Z., Wang, P., Yu, J., Wan, L., Chen, J., Xiao, M., 2011. Characterisation of interaction between NS3 and NS5B protein of classical swine fever virus by deletion of terminal sequences of NS5B. Virus Res. 156 (1–2), 98–106.
Wang, Z., Nie, Y., Wang, P., Ding, M., Deng, H., 2004. Characterization of classical swine fever virus entry by using pseudotyped viruses: E1 and E2 are sufficient to mediate viral entry. Virology 330 (1), 332–341.
Watanabe, T., Wang, S., Noritake, J., Sato, K., Fukata, M., Takefuji, M., Nakagawa, M., Izumi, N., Akiyama, T., Kaibuchi, K., 2004. Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration. Dev. Cell 7 (6), 871–883.
Weiland, E., Ahl, R., Stark, R., Weiland, F., Thiel, H.-J., 1992. A second envelope glycoprotein mediates neutralization of a pestivirus, hog cholera virus. J. Virol. 66 (6), 3677–3682.
Weiland, E., Stark, R., Haas, B., Rümenapf, T., Meyers, G., Thiel, H.-J., 1990. Pestivirus glycoprotein which induces neutralizing antibodies forms part of a disulfide-linked heterodimer. J. Virol. 64 (8), 3563–3569.
Wen, G., Chen, C., Luo, X., Wang, Y., Zhang, C., Pan, Z., 2007. Identification and characterization of the NTPase activity of classical swine fever virus (CSFV) nonstructural protein 3 (NS3) expressed in bacteria. Arch. Virol. 152 (8), 1565–1573.
Wen, G., Xue, J., Shen, Y., Zhang, C., Pan, Z., 2009. Characterization of classical swine fever virus (CSFV) nonstructural protein 3 (NS3) helicase activity and its modulation by CSFV RNA-dependent RNA polymerase. Virus Res. 141 (1), 63–70.
Wensvoort, G., Boonstra, J., Bodzinga, B., 1990. Immunoaffinity purification and characterization of the envelope protein E1 of hog cholera virus. J. Gen. Virol. 71 (3), 531–540.
Wong, J., Zhang, J., Si, X., Gao, G., Mao, I., McManus, B.M., Luo, H., 2008. Autophagosome supports coxsackievirus B3 replication in host cells. J. Virol. 82 (18), 9143–9153.
Xiao, M., Bai, Y., Xu, H., Geng, X., Chen, J., Wang, Y., Chen, J., Li, B., 2008. Effect of NS3 and NS5B proteins on classical swine fever virus internal ribosome entry site-mediated translation and its host cellular translation. J. Gen. Virol. 89 (4), 994–999.
Xiao, M., Gao, J., Wang, W., Wang, Y., Chen, J., Chen, J., Li, B., 2004. Specific interaction between the classical swine fever virus NS5B protein and the viral genome. Eur. J. Biochem. 271 (19), 3888–3896.
Xiao, M., Li, H., Wang, Y., Wang, X., Wang, W., Peng, J., Chen, J., Li, B., 2006. Characterization of the N-terminal domain of classical swine fever virus RNA-dependent RNA polymerase. J. Gen. Virol. 87 (2), 347–356.
Xiao, M., Wang, Y., Zhu, Z., Yu, J., Wan, L., Chen, J., 2009. Influence of NS5A protein of classical swine fever virus (CSFV) on CSFV internal ribosome entry site-dependent translation. J. Gen. Virol. 90 (12), 2923–2928.
Xiao, M., Zhang, C., Pan, Z., Wu, H., Guo, J., 2002. Classical swine fever virus NS5B-GFP fusion protein possesses an RNA-dependent RNA polymerase activity. Arch. Virol. 147 (9), 1779–1787.
Xu, J., Mendez, E., Caron, P.R., Lin, C., Murcko, M.A., Collett, M.S., Rice, C.M., 1997. Bovine viral diarrhea virus NS3 serine proteinase: polyprotein cleavage sites, cofactor requirements, and molecular model of an enzyme essential for pestivirus replication. J. Virol. 71 (7), 5312–5322.
Yang, X.-J., Liu, J., Ye, L., Liao, Q.-J., Wu, J.-G., Gao, J.-R., She, Y.-L., Wu, Z.-H., Ye, L.-B., 2006. HCV NS2 protein inhibits cell proliferation and induces cell cycle arrest in the S-phase in mammalian cells through down-regulation of cyclin A expression. Virus Res. 121 (2), 134–143.
Yin, L., Luo, Y., Liang, B., Wang, F., Du, M., Petrenko, V.A., Qiu, H.-J., Liu, A., 2014. Specific ligands for classical swine fever virus screened from landscape phage display library. Antivir. Res. 109, 68–71.
Yueh, A., Leung, J., Bhattacharyya, S., Perrone, L.A., de los Santos, K., Pu, S.-y., Goff, S.P., 2006. Interaction of Moloney murine leukemia virus capsid with Ubc9 and PIASy mediates SUMO-1 addition required early in infection. J. Virol. 80 (1), 342–352.

Zhou, T., 1980. Progress of classical swine fever virus and the prevention of the disease (partI). Chin. J. Vet. Sci. Technol. 4, 23–33.
Zhao, Y., Pang, D., Wang, T., Yang, X., Wu, R., Ren, L., Yuan, T., Huang, Y., Ouyang, H., 2011. Human MxA protein inhibits the replication of classical swine fever virus. Virus Res. 156 (1–2), 151–155.
Zhou, J.-f., Hua, X.-g., Cui, L., Zhu, J.-g., Miao, D.-n., Zou, Y., He, X.-z., Su, W.-g., 2007. Effective inhibition of porcine transmissible gastroenteritis virus replication in ST cells by shRNAs targeting RNA-dependent RNA polymerase gene. Antivir. Res. 74 (1), 36–42.
Zhou, Z., Jiang, X., Liu, D., Fan, Z., Hu, X., Yan, J., Wang, M., Gao, G.F., 2009. Autophagy is involved in influenza A virus replication. Autophagy 5 (3), 321–328.
Zhu, Z., Wang, Y., Yu, J., Wan, L., Chen, J., Xiao, M., 2010. Classical swine fever virus NS3 is an IRES-binding protein and increases IRES-dependent translation. Virus Res. 153 (1), 106–112.
Zou, X.-q., Zhao, Q.-z., Fan, Y.-f., Zhu, Y.-y., Wang, Q., Xu, L., Fan, X.-z., Ning, Y.-b., 2011. Construction of the full length infectious cDNA clones of CSFV C strain and virus rescue. Sci. Agric. Sin. 2, 023.