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MINIREVIEW

Overview: Replication of Porcine Reproductive and Respiratory Syndrome Virus

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Porcine reproductive and respiratory syndrome virus (PRRSV), an arterivirus that causes significant losses in the pig industry, is one of the most important animal pathogens of global significance. Since the discovery of the virus, significant progress has been made in understanding its epidemiology and transmission, but no adequate control measures are yet available to eliminate infection with this pathogen. The genome replication of PRRSV is required to reproduce, within a few hours of infection, the millions of progeny virions that establish, disseminate, and maintain infection. Replication of the viral RNA genome is a multistep process involving a replication complex that is formed not only from components of viral and cellular origin but also from the viral genomic RNA template; this replication complex is embedded within particular virus-induced membrane vesicles. PRRSV RNA replication is directed by at least 14 replicase proteins that have both common enzymatic activities, including viral RNA polymerase, and also unusual and poorly understood RNA-processing functions. In this review, we summarize our current understanding of PRRSV replication, which is important for developing a successful strategy for the prevention and control of this pathogen.

Keywords: porcine reproductive and respiratory syndrome virus, RNA replication, viral gene expression, arterivirus

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of PRRS (Terpstra *et al.*, 1991; Wensvoort *et al.*, 1991; Benfield *et al.*, 1992; Collins *et al.*, 1992), an economically devastating, pandemic disease of swine that is typically characterized by reproductive failure in breeding herds and respiratory problems and growth retardation in growing pigs (Done and Paton, 1995; Botner, 1997; Van Reeth, 1997; Zimmerman *et al.*, 1997; Rossow, 1998; Suarez, 2000; Rowland, 2010). Two PRRS outbreaks were first reported in the late 1980s in North America (Keffaber, 1989; Hill, 1990) and central Europe (Paton *et al.*, 1991). The disease is now found in most pig-producing countries and affects the swine industry and food safety worldwide (Albina, 1997; Blaha, 2000; Lunney *et al.*, 2010; Shi *et al.*, 2010a), causing enormous economic losses each year (Brouwer *et al.*, 1994; Garner *et al.*, 2001; Zimmerman *et al.*, 2006; Nieuwenhuis *et al.*, 2012). In the US, the annual loss due to PRRS is estimated to exceed $500 million (Neumann *et al.*, 2005). In particular, the emergence of highly pathogenic PRRSVs in China and Vietnam in 2006 (Li *et al.*, 2007; Tian *et al.*, 2007; Feng *et al.*, 2008; Zhou *et al.*, 2008; An *et al.*, 2010b) and their rapid spread to several neighboring Asian countries (An *et al.*, 2011) have raised a growing concern that new pathogenic PRRSVs can spread throughout the world, posing a significant threat to the global agricultural community (Normile, 2007; Lunney and Chen, 2010; Murtaugh *et al.*, 2010; Zhou and Yang, 2010). Because of the current burden of PRRS and the emergence of highly pathogenic forms of PRRSV on a global level, control of this virus remains a research priority in all pig-producing countries.

Classification

PRRSV belongs to the family *Arteriviridae* in the order *Nidovirales*, which also includes two other families, the *Coronaviridae* and *Roniviridae* (Gorbalenya *et al.*, 2006; de Groot *et al.*, 2011). Within the *Arteriviridae* family, PRRSV forms a single genus *Arterivirus*, together with equine arteritis virus (EAV), lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus (Plagemann and Moennig, 1992; Cavanagh, 1997; de Vries *et al.*, 1997; Faaberg *et al.*, 2011). Like other arteriviruses, PRRSV is an enveloped virus (Dokland, 2010) containing a non-isometric nucleocapsid core (Spilman *et al.*, 2009) that encapsidates a plus-strand genomic RNA of ~15 kb in length (Meulenberg *et al.*, 1993). This genomic RNA consists of a 5′-untranslated region (UTR), 10 open reading frames (ORFs), and a 3′-UTR (Snijder and Spaan, 2007; Britton and Cavanagh, 2008; Firth *et al.*, 2011; Johnson *et al.*, 2011) (Fig. 1).

Genetic heterogeneity

Based on its genetic diversity and geographic distribution, PRRSV is divided into two major genotypes (Murtaugh *et al.*,

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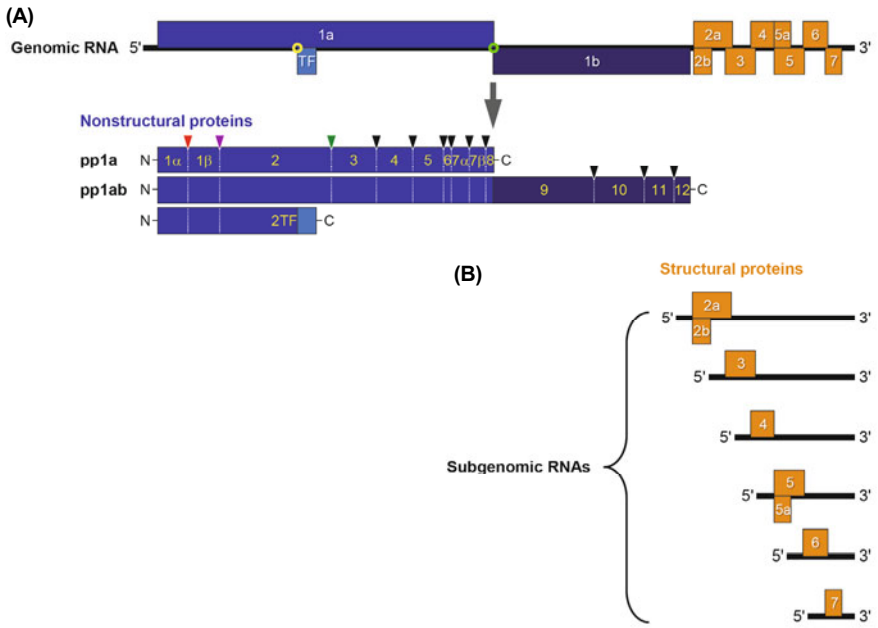

Fig. 1. Expression of the PRRSV genomic RNA. (A) Synthesis of the viral nonstructural proteins (NSPs) from the genomic RNA. The ~15-kb plus-strand genomic RNA of PRRSV is shown on top. Two long 5′-proximal ORFs (ORF1a and ORF1b) are translated into two large polyprotein precursors, pp1a and pp1ab; the latter is synthesized by a -1 ribosomal frameshift. The two polyproteins are cleaved into at least 14 NSPs: 10 encoded in ORF1a (NSP1α, NSP1β, NSP2 to NSP6, NSP7α, NSP7β, and NSP8) and 4 encoded in ORF1b (NSP9 to NSP12). This proteolysis is regulated by four viral proteases, namely NSP1α, NSP1β, NSP2, and NSP4. An additional protein designated NSP2TF is translated by a -2 ribosomal frameshift in the NSP2-coding region. (B) Synthesis of the viral structural proteins from the six subgenomic mRNAs. Eight short 3′-proximal ORFs are translated from a nested set of six major subgenomic mRNAs: ORF2a (GP2/2a), ORF2b (E, envelope), ORF3 (GP3), ORF4 (GP4), ORF5 (GP5), ORF6 (M, membrane), ORF7 (N, nucleocapsid), and a newly discovered protein encoded in ORF5a that overlaps with the 5′-end of ORF5.

2010; Shi *et al.*, 2010a): Type 1, represented by the European prototype Lelystad strain (Wensvoort *et al.*, 1991); and Type 2, exemplified by the North American prototype VR-2332 strain (Benfield *et al.*, 1992; Collins *et al.*, 1992). Interestingly, despite their concurrent emergence and similar clinical symptoms (Halbur *et al.*, 1995), the two genotypes show ~40% genetic divergence (Mardassi *et al.*, 1994; Kapur *et al.*, 1996; Allende *et al.*, 1999; Nelsen *et al.*, 1999; Meng, 2000; Oleksiewicz *et al.*, 2000; Forsberg *et al.*, 2002), with a high degree of antigenic variation (Wensvoort *et al.*, 1992; Nelson *et al.*, 1993; Drew *et al.*, 1995; Wootton *et al.*, 1998). Over the last decade, this genetic/antigenic diversity has expanded continuously and rapidly (Murtaugh *et al.*, 2001; Stadejek *et al.*, 2002; Mateu *et al.*, 2003; Pesch *et al.*, 2005; Han *et al.*, 2006; Stadejek *et al.*, 2006, 2008; Balka *et al.*, 2008; Li *et al.*, 2009, 2011; Shi *et al.*, 2010b), highlighting the dynamic nature of PRRSV evolution and epidemiology. At present, a larger number of genetically heterogeneous PRRSVs are widely co-circulating throughout the world than ever before (Dewey *et al.*, 2000; Goldberg *et al.*, 2003; Ropp *et al.*, 2004; Thanawongnuwech *et al.*, 2004; Fang *et al.*, 2007), posing a significant challenge for the diagnosis, prevention, and control of PRRSV infection.

Transmission

PRRSV is transmitted both horizontally (pig-to-pig infection) and vertically (transplacental infection) to fetuses during mid-to-late gestation (Christianson *et al.*, 1992, 1993; Yaeger *et al.*, 1993); horizontal transmission occurs through both direct and indirect contact (Cho and Dee, 2006; Zimmerman *et al.*, 2006). Direct contact is the most efficient route of PRRSV transmission, via a variety of porcine secretions from infected animals in which the virus has been detected: e.g., saliva (Wills *et al.*, 1997a; Prickett *et al.*, 2008), milk (Wagstrom *et al.*, 2001), nasal fluids (Rossow *et al.*, 1994), and semen (Swenson *et al.*, 1994; Christopher-Hennings *et al.*, 1995). Although its mechanism(s) remains elusive (Mateu and Diaz, 2008; Lunney and Chen, 2010; Yoo *et al.*, 2010; Murtaugh and Genzow, 2011), PRRSV persistence in pigs plays an important role in viral transmission because the virus is present at low levels in the infected animals (Wills *et al.*, 1997b, 2003; Allende *et al.*, 2000; Bierk *et al.*, 2001; Batista *et al.*, 2002, 2004; Horter *et al.*, 2002). In addition to these direct routes of PRRSV transmission, indirect routes of a particular concern include contaminated fomites (Dee *et al.*, 2002, 2003; Otake *et al.*, 2002b), needles (Otake *et al.*, 2002c), transport vehicles (Dee *et al.*, 2004), aerosols (Torremorell *et al.*, 1997; Brockmeier and Lager, 2002; Mortensen *et al.*, 2002; Otake *et al.*, 2002a, 2010; Kristensen *et al.*, 2004; Trincado *et al.*, 2004; Fano *et al.*, 2005; Dee *et al.*, 2009; Pitkin *et al.*, 2009), and insects as a mechanical vector (Otake *et al.*, 2002d, 2003a, 2003b; Schurrer *et al.*, 2004, 2005).

Replication cycle

PRRSV infection is initiated by the attachment of the virions to the highly sulfated, negatively charged glycosaminoglycans on the surface of susceptible cells (Jusa *et al.*, 1997; Vanderheijden *et al.*, 2001; Delputte *et al.*, 2002), followed by binding to CD169 (Duan *et al.*, 1998a, 1998b; Vanderheijden *et al.*, 2003; Delputte and Nauwynck, 2004; Delputte *et al.*, 2005, 2007; An *et al.*, 2010a; Van Breedam *et al.*, 2010b), which triggers receptor-mediated clathrin-dependent endocytosis (Kreutz and Ackermann, 1996; Nauwynck *et al.*, 1999; Vanderheijden *et al.*, 2003). At the early endosomes, the viral genome is released into the cytoplasm through a reaction mediated by CD163 (Calvert *et al.*, 2007; Van Gorp *et al.*, 2008, 2009; Das *et al.*, 2010; Van Gorp *et al.*, 2010) and presumably other cellular factors (Misinzo *et al.*, 2008).

Once the genome enters the cytoplasm, ORF1a and ORF1b, located in the 5′-proximal three-quarters of the viral genome,

are translated to produce two large polyproteins, pp1a and pp1ab (Snijder and Meulenberg, 1998; Snijder and Spaan, 2007), with the expression of pp1ab controlled by a -1 ribosomal frameshift (Brierley *et al*., 1989; den Boon *et al*., 1991) (Fig. 1A). Autocatalytic processing of these precursors generates at least 14 nonstructural proteins (NSPs) (Ziebuhr *et al*., 2000; Van Hemert and Snijder, 2008; Fang and Snijder, 2010): 10 encoded in ORF1a (NSP1α, NSP1β, NSP2 to NSP6, NSP7α, NSP7β, and NSP8) and 4 encoded in ORF1b (NSP9 to NSP12) (Snijder *et al*., 1992, 1994; den Boon *et al*., 1995; van Dinten *et al*., 1996; Wassenaar *et al*., 1997; Chen *et al*., 2010a; Li *et al*., 2012). This proteolytic processing is mediated by four viral proteases residing in NSP1α, NSP1β, NSP2, and NSP4 (den Boon *et al*., 1995; Snijder *et al*., 1996; van Aken *et al*., 2006b). Also, an additional viral protein is synthesized by a -2 ribosomal frameshift in the NSP2-coding region, yielding a transframe fusion NSP2TF with the N-terminal two-thirds of NSP2 (Fang *et al*., 2012). Most, if not all, of the NSPs assemble into a replication and transcription complex (RTC) that accumulates at the virus-induced ER-derived double-membrane vesicles (van der Meer *et al*., 1998; Pedersen *et al*., 1999; Kroese *et al*., 2008). The RTC directs both genome amplification ("replication") and subgenomic mRNA synthesis ("transcription") (Fang and Snijder, 2010); the latter, a hallmark of all nidoviruses (Pasternak *et al*., 2006; Sawicki *et al*., 2007; Snijder and Spaan, 2007), produces a nested set of six major subgenomic mRNAs that are both 5′- and 3′-coterminal with the genomic RNA and thus consist of nucleotide sequences that are non-contiguous in the genomic RNA (de Vries *et al*., 1990).

Through the six subgenomic mRNAs, eight mostly overlapping ORFs situated in the 3′-proximal region of the viral genome are expressed, presumably by utilizing each subgenomic mRNA for the translation of one or two of its most 5′-proximal ORFs (Conzelmann *et al*., 1993; Meng *et al*., 1996) (Fig. 1B). These ORFs encode eight structural proteins that constitute the infectious virion (Snijder and Meulenberg, 1998; Snijder and Spaan, 2007; Dokland, 2010): i.e., four minor components encoded in ORF2a (GP2/2a), ORF2b (E, envelope), ORF3 (GP3), and ORF4 (GP4); three major components encoded in ORF5 (GP5), ORF6 (M, membrane), and ORF7 (N, nucleocapsid) (Meulenberg *et al*., 1995; Meulenberg and Petersen-den Besten, 1996; van Nieuwstadt *et al*., 1996; Snijder *et al*., 1999; Wu *et al*., 2001); and a recently identified protein encoded in ORF5a that overlaps with the 5′-end of ORF5 (Firth *et al*., 2011; Johnson *et al*., 2011).

At the late stage of viral replication, multiple copies of the N proteins bind to the newly synthesized genomic RNA to form a nucleocapsid complex (Tijms *et al*., 2002), which buds into the lumen of the smooth ER and/or Golgi complex (Wood *et al*., 1970; Stueckemann *et al*., 1982; Dea *et al*., 1995; Weiland *et al*., 1995; Pol *et al*., 1997) and acquires the six viral envelope proteins, i.e., E, M, and GP2 to GP5 (Snijder *et al*., 2003b; Wieringa *et al*., 2004; Zevenhoven-Dobbe *et al*., 2004; Wissink *et al*., 2005). In this event, the role of the protein product of ORF5a is unclear. Finally, the progeny virions accumulated in the intracellular membrane compartments are released into the extracellular space through exocytosis (Dea *et al*., 1995).

Viral nonstructural proteins

Although considerable research has been focused on PRRSV, little is known about the proteolytic processing pathway and the structure and function of most of the PRRSV NSPs. The initial functional assignments of the PRRSV NSPs have primarily been based on the experimental data of EAV, the arterivirus prototype (Fig. 1A).

NSP1α and NSP1β: PRRSV NSP1α and NSP1β each contain a cysteine protease domain responsible for autocatalytic processing at the NSP1α/1β (den Boon *et al*., 1995; Sun *et al*., 2009; Chen *et al*., 2010a) and NSP1β/2 (den Boon *et al*., 1995; Chen *et al*., 2010a) junctions, respectively. The atomic structure of PRRSV NSP1α reveals three domains (Sun *et al*., 2009): (*i*) a N-terminal zinc-finger domain, (*ii*) a papain-like cysteine protease (PCPα) domain with a zinc ion bound at the active site that is required for its proteolytic activity, and (*iii*) a C-terminal extension bound to the substrate binding site of the PCPα domain. Similarly, the crystal structure of PRRSV NSP1β reveals four domains (Xue *et al*., 2010): (*i*) an N-terminal metal-dependent nuclease domain, (*ii*) a linker domain, (*iii*) a papain-like cysteine protease (PCPβ) domain, and (*iv*) a C-terminal extension bound to the substrate binding site of the PCPβ domain, as observed for PRRSV NSP1α. In the case of both NSP1α and NSP1β, their C-terminal extensions occupy the protease active site after their release from the polyprotein, suggesting that they function *in cis* (Sun *et al*., 2009; Xue *et al*., 2010). In PRRSV, inactivation of the PCPα activity in NSP1α blocks subgenomic mRNA synthesis without altering genome replication, whereas when PCPβ activity is eliminated in NSP1β, no sign of viral RNA synthesis is seen; therefore, both PCP protease activities are apparently required for productive viral RNA synthesis (Kroese *et al*., 2008). Similarly, mutagenesis studies have shown that EAV NSP1 (which contains a tandem of the PCPα and PCPβ domains, with PCPα having lost its enzymatic activity) is involved in regulating the accumulation of minus-strand templates to control the relative abundance of viral mRNAs, thereby coordinating genome replication, subgenomic mRNA synthesis, and virus production (Tijms *et al*., 2001, 2007; Nedialkova *et al*., 2010). Both PRRSV NSP1α/1β (Chen *et al*., 2010a) and EAV NSP1 (Tijms *et al*., 2002) are translocated to the nucleus in infected cells, but no consensus nuclear localization signal has yet been found. The interaction of EAV NSP1 with the cellular transcription co-factor p100 suggests that it might be important for viral and/or cellular transcription (Tijms and Snijder, 2003).

NSP2 and NSP3: PRRSV NSP2 is predicted to have four domains: (*i*) an N-terminal cysteine protease domain, (*ii*) a large hypervariable domain, (*iii*) a transmembrane domain, and (*iv*) a C-terminal tail (Han *et al*., 2009). The cysteine protease belongs to the mammalian ovarian tumor domain (OTU)-containing protein superfamily (Makarova *et al*., 2000; Han *et al*., 2009); it cleaves at the NSP2/3 junction that functions both *in cis* and *in trans* (Snijder *et al*., 1995; Han *et al*., 2009) and is crucial for the viral replication cycle (Han *et al*., 2009). In EAV-infected cells, NSP2 is localized to the perinuclear membranes, which are presumably derived from the ER and are involved in the formation of the membrane-bound RTC, where viral RNA synthesis occurs (van der Meer *et al*., 1998; Pedersen *et al*., 1999). In the ab-

sence of EAV replication, the co-expression of EAV NSP2 and NSP3 is both necessary and sufficient to modify host cell membranes during the formation of the RTC (Snijder *et al*., 2001). Also, EAV NSP2 interacts with NSP3 (Snijder *et al*., 1994), and NSP3 has been implicated in the process of remodeling intracellular membranes (Posthuma *et al*., 2008). Biochemical and morphologic studies of EAV replication have shown that the NSPs containing transmembrane domains (e.g., NSP2, NSP3, and NSP5) are part of the membrane-bound RTC, suggesting that they play an important role in recruiting other viral components of the RTC that lack the membrane-spanning domains (van der Meer *et al*., 1998). *In vitro*, the EAV RTCs isolated from infected cells require a cytosolic host factor for viral RNA synthesis, which reproduces the synthesis of both viral genome and subgenomic mRNAs (van Hemert *et al*., 2008). Interestingly, PRRSV NSP2 contains a cluster of linear B-cell epitopes that are dispensable for virus replication (Oleksiewicz *et al*., 2001; Chen *et al*., 2010b) but capable of modulating the host immune response (Chen *et al*., 2010b; Li *et al*., 2010).

NSP4: PRRSV NSP4 contains the main protease (3C-like serine proteinase) (Snijder *et al*., 1996) responsible for all NSP processing, except for the NSP1α/1β, NSP1β/2, and NSP2/3 junctions (van Dinten *et al*., 1999; Ziebuhr *et al*., 2000). Cleavages at the NSP3/4, NSP4/5, and NSP11/12 junctions have been confirmed experimentally by the use of recombinant PRRSV NSP4 (Tian *et al*., 2009). The crystal structure of both PRRSV and EAV NSP4s reveals a chymotrypsin-like fold with a canonical catalytic triad (S-H-D), as well as a novel α/β C-terminal extension (Barrette-Ng *et al*., 2002; Tian *et al*., 2009) that may be involved in regulating viral polyprotein processing (van Aken *et al*., 2006a).

NSP9: Arterivirus NSP9 includes the viral RNA-dependent RNA polymerase (RdRp) (den Boon *et al*., 1991). In PRRSV, the RdRp domain is located in the C-terminal region, which contains an upstream N-terminal region of unknown function (Gorbalenya *et al*., 2006; Fang and Snijder, 2010). Enzymatically active EAV RdRp can be purified from *E. coli* and initiates RNA synthesis by a *de novo* mechanism on homopolymeric templates in a template-dependent fashion (Beerens *et al*., 2007).

NSP10: PRRSV NSP10 is predicted to have three domains (Gorbalenya *et al*., 2006; Fang and Snijder, 2010): (*i*) an N-terminal zinc-binding domain, (*ii*) a linker domain, and (*iii*) a nucleotide triphosphate binding or helicase domain (den Boon *et al*., 1991). Bacterially expressed PRRSV and EAV NSP10s possess ATPase activity and can unwind dsRNA and dsDNA in a 5′-to-3′ direction (Bautista *et al*., 2002; Seybert *et al*., 2005). The zinc-binding domain of EAV NSP10 is also critical for this activity (Seybert *et al*., 2005). In EAV, the zinc-binding domain contains a set of 13 conserved Cys and His residues and is critical for viral RNA synthesis (van Dinten *et al*., 2000). The linker domain (S2429P) has been implicated in subgenomic mRNA synthesis (van Dinten *et al*., 1997; van Marle *et al*., 1999b).

NSP11: Arterivirus NSP11 contains the uridylate-specific endoribonuclease (NendoU) domain, which is a major genetic marker unique to nidoviruses (Snijder *et al*., 2003a; Gorbalenya *et al*., 2006; Fang and Snijder, 2010). Bacterially expressed NSP11 has been used to show that the endoribonuclease activity of both PRRSV and EAV NendoUs exhibits broad substrate specificity *in vitro*, but its function in infected cells is elusive (Nedialkova *et al*., 2009). Viruses with mutations in the EAV NendoU active site are viable but have a defect in subgenomic mRNA synthesis (Posthuma *et al*., 2006). Recently, IFN-mediated host innate immunity has been shown to be modulated by a panel of PRRSV NSPs (i.e., NSP1α, NSP1β, NSP2, NSP4, and NSP11) with different intensities (Beura *et al*., 2010; Chen *et al*., 2010a; Li *et al*., 2010). In the case of PRRSV NSP2, the OTU domain-containing cysteine protease has been shown to possess deubiquitinating and interferon antagonism activity, thereby evading ubiquitin- and ISG15-dependent innate immunity (Frias-Staheli *et al*., 2007; Sun *et al*., 2010).

Other NSPs: To date, no specific functions have been demonstrated for the other PRRSV NSPs (NSP5, NSP6, NSP7α, NSP7β, NSP8, and NSP12). Also, it should be noted that during the proteolytic processing of EAV NSPs, many cleavage intermediates of unknown function have been observed (Snijder *et al*., 1994; van Dinten *et al*., 1996), and alternative major and minor processing pathways have also been characterized (Wassenaar *et al*., 1997).

Viral structural proteins

Based on the "discontinuous RNA transcription" model (Sawicki and Sawicki, 1995), the plus-strand genomic RNA of PRRSV is thought to serve as a template for either (*i*) continuous minus-strand RNA synthesis, which produces the genome-length minus-strand template for genome replication; or (*ii*) discontinuous minus-strand RNA synthesis, which generates a nested set of six major subgenome-length minus-strand templates, one for each subgenomic mRNA synthesis. All the subgenomic mRNAs are both 5′- and 3′-coterminal with the genomic RNA, with a common short "leader" sequence corresponding to the 5′-proximal region of the genome joined to different "body" segments that are co-linear with its 3′-proximal region (Pasternak *et al*., 2006; Sawicki *et al*., 2007). This leader-body joining is guided by regulatory transcription-regulating sequences (TRSs); in the genomic RNA, these RNA motifs are located at the 3′-end of the leader sequence (leader TRS) and upstream of each structural protein-coding region (body TRS) (van Marle *et al*., 1999a; Pasternak *et al*., 2001; Van Den Born *et al*., 2004). In PRRSV, the 5′-proximal one or two ORFs of each subgenomic mRNA are translated to produce eight viral structural proteins that constitute an infectious virion (Meulenberg *et al*., 1995; Meulenberg and Petersen-den Besten, 1996; van Nieuwstadt *et al*., 1996; Snijder *et al*., 1999; Dea *et al*., 2000; Molenkamp *et al*., 2000; Wu *et al*., 2001; Johnson *et al*., 2011): GP2 (GP2a), E, GP3, GP4, GP5, M, N, and a protein product of ORF5a (Fig. 1B).

The viral envelope contains the two major (GP5 and M) and four minor (E, GP2, GP3, and GP4) membrane proteins that are all required for the production and infectivity of infectious virions; however, the four minor proteins are dispensable for virus assembly (Wieringa *et al*., 2004; Wissink *et al*., 2005). E protein has an ion channel protein-like property and is embedded in the viral membrane, presumably promoting uncoating of the virion and release of the viral genome into the cytoplasm (Lee and Yoo, 2006). GP3 is

heavily glycosylated (Dea *et al*., 2000; Das *et al*., 2011), and its glycans on the viral surface prevent the recognition of epitopes by neutralizing antibodies (Vu *et al*., 2011); a subset of the GP3 proteins is secreted from the cells as a non-virion-associated soluble form (Mardassi *et al*., 1998). GP4 has a neutralizing epitope in the hypervariable region (Meulenberg *et al*., 1997) that might be associated with the E, GP2, and GP3 proteins through non-covalent interactions (Wieringa *et al*., 2004; Wissink *et al*., 2005; Das *et al*., 2010). GP5 is a triple membrane-spanning protein with a short ectodomain (~40 aa) and a long cytoplasmic tail (~50–70 aa) (Meulenberg *et al*., 1995; Mardassi *et al*., 1996), which contains major neutralizing epitopes (Wissink *et al*., 2003; Ansari *et al*., 2006). M is the most conserved membrane protein and has a membrane topology similar to that of GP5 (Dea *et al*., 2000). N is a serine phosphoprotein that forms a dimer and is distributed in the cytoplasm and the nucleus (Rowland and Yoo, 2003; You *et al*., 2008). In the viral membrane, the GP5 and M proteins are embedded as disulfide-linked heterodimers, whereas the E, GP2, GP3, and GP4 proteins are associated with each other through non-covalent interactions (Meulenberg *et al*., 1993, 1995; Mardassi *et al*., 1995, 1996; Meulenberg and Petersen-den Besten, 1996; van Nieuwstadt *et al*., 1996; Wu *et al*., 2001; Wissink *et al*., 2005).

PRRSV has a very restricted cell tropism. *In vivo*, it targets specific subsets of porcine macrophages, primarily alveolar macrophages (Lawson *et al*., 1997; Duan *et al*., 1997a, 1997b; Teifke *et al*., 2001); *in vitro*, it can also infect monocyte- or bone marrow-derived porcine dendritic cells when stimulated with GM-CSF/IL-4 (Loving *et al*., 2007; Wang *et al*., 2007; Chang *et al*., 2008; Flores-Mendoza *et al*., 2008; Silva-Campa *et al*., 2009), but not lung dendritic cells (Loving *et al*., 2007). PRRSV entry into porcine macrophages is the first step in a highly coordinated process of virus-host interactions. Based on recent findings (Welch and Calvert, 2010; Van Breedam *et al*., 2010a), highly sulfated, negatively charged glycosaminoglycans such as heparan sulfates can be used as low-affinity attachment factors that concentrate virus particles on the cell surface (Jusa *et al*., 1997; Vanderheijden *et al*., 2001; Delputte *et al*., 2002, 2005). Once this interaction has taken place, the viral GP5/M complex binds to the N-terminal portion of CD169 (also called sialoadhesin or siglec-1) (Duan *et al*., 1998a, 1998b; Vanderheijden *et al*., 2003; Delputte *et al*., 2005; Van Gorp *et al*., 2008; An *et al*., 2010a; Van Breedam *et al*., 2010b). This interaction is directed by the sialic acid-binding domain at the N-terminus of CD169 and sialic acids on the virion surface (Delputte and Nauwynck, 2004; Delputte *et al*., 2007; Van Breedam *et al*., 2010b), which trigger receptor-mediated, clathrin-dependent endocytosis (Kreutz and Ackermann, 1996; Nauwynck *et al*., 1999; Vanderheijden *et al*., 2003). Once internalized, the particles are transported to early endosomes, where the viral genome is released into the cytoplasm in a reaction that depends on both the acidic environment and scavenger receptor CD163 (Nauwynck *et al*., 1999; Calvert *et al*., 2007; Van Gorp *et al*., 2008, 2009). The role of CD163 is mediated through its cysteine-rich domain 5 (Van Gorp *et al*., 2010) and by interaction with GP2 and GP4 (Das *et al*., 2010). The protease cathepsin E and an additional serine protease are also implicated in this process (Misinzo *et al*., 2008). Other host factors, such as simian vimentin (Kim *et al*., 2006) and CD151 (Shanmukhappa *et al*., 2007), have been identified in MARC-145 cells, a cell line susceptible to PRRSV infection (Kreutz, 1998).

Vaccines

PRRS is controlled by several different strategies, including management (e.g., herd depopulation/repopulation, herd closure, and regional elimination), biosecurity, and vaccination (Corzo *et al*., 2010; Thanawongnuwech and Suradhat, 2010). Of these strategies, vaccination is the most cost-effective for controlling PRRS, but it does not completely prevent PRRSV infection. Two types of PRRSV vaccines are commercially available: modified-live virus (MLV) and killed virus (KV) vaccines (Yoo *et al*., 2004; Charerntantanakul, 2009; Kimman *et al*., 2009; Cruz *et al*., 2010; Huang and Meng, 2010). The MLV vaccine confers effective protection against genetically homologous PRRSVs but only partial protection against genetically heterologous PRRSVs (Meng, 2000; Murtaugh *et al*., 2002; Labarque *et al*., 2003; Okuda *et al*., 2008); it is of particular concern that the live vaccine viruses have the potential to spontaneously revert to virulence and spread the disease (Botner *et al*., 1997; Madsen *et al*., 1998; Mengeling *et al*., 1999; Storgaard *et al*., 1999; Wesley *et al*., 1999; Nielsen *et al*., 2001; Opriessnig *et al*., 2002; Amonsin *et al*., 2009; grosse Beilage *et al*., 2009; Li *et al*., 2009). The KV vaccine, on the other hand, is safe but offers limited protection at best against either homologous or heterologous PRRSVs (Scortti *et al*., 2007; Zuckermann *et al*., 2007; Vanhee *et al*., 2009). Thus, the current vaccines fail to provide sustainable disease control and prevention, particularly against the genetically heterologous PRRSVs (Cano *et al*., 2007a, 2007b), making it difficult to achieve global eradication.

Conclusion

Although significant progress has been made in understanding the routes of PRRSV transmission and in developing and implementing control measures for PRRSV infection, there is clearly an urgent need for novel strategies that may be applicable to the development of a safer, more effective vaccine against PRRSV. Despite the clinical importance of PRRSV in animal health, only limited information is available to date regarding the biological functions of the viral nonstructural and structural proteins in replication and pathogenesis. In particular, the molecular characterization of the 14 replicase proteins and their roles in PRRSV RNA synthesis have represented a major challenge in PRRSV biology. We and others have established a reverse genetics system for PRRSV by constructing a full-length infectious cDNA that allows genetic manipulation of the viral genome and from which molecularly cloned viruses can be rescued. This system offers a unique opportunity to address some of the key questions in PRRSV biology. New information will give us a better understanding of the molecular and genetic basis of PRRSV replication and pathogenesis, a prerequisite for the development of new and promising strategies to

control and eliminate this pathogen.

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