



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

MOLECULAR BIOLOGY OF BOVINE VIRAL DIARRHEA VIRUS AND ITS INTERACTIONS WITH THE HOST

Ruben O. Donis, MV, PhD

The viral etiology of a clinical disease in cattle, which was caused by a transmissible agent that later became bovine viral diarrhea virus (BVDV), was established by Olafson et al 50 years ago.⁹¹ Early work confirmed BVDV as a novel viral entity, distinct from rinderpest and other exotic viruses.⁹² The close relationship between this agent and hog cholera virus (also known as classical swine fever virus), then still enzootic in the United States, did not become apparent until the 1960s.^{31, 37} A decade later, an ovine virus, the causative agent of border disease, was identified as a member of this group.⁹⁸ The term *pestivirus* was coined by Horzinek to refer to this group of viruses. At the time, pestiviruses were considered an oddity among togaviruses not transmitted by arthropods.^{65, 102} Pestiviruses owe their new affiliation, separate from the togaviridae, to nucleotide sequence of their genomic RNA provided by Collett et al and Meyers et al in the last decade.^{26, 79} Since then, the contribution of molecular genetic research to our understanding of the biology of the virus at the cellular and organismal levels has been invaluable.²⁵ Taxonomic uncertainties surrounding the classification

Research in the author's laboratory was supported by grant funds from the United States Department of Agriculture NRI Agreement no. 92-37204-7959, the Center for Biotechnology of the University of Nebraska, and the Layman Foundation.

From the Department of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska

VETERINARY CLINICS OF NORTH AMERICA: FOOD ANIMAL PRACTICE

of BVDV and related pestiviruses of sheep and swine within togaviridae or flaviviridae have been clarified, resulting in the definitive assignment of *Pestivirus* as a genus of flaviviridae. Space considerations will limit this far from comprehensive review to an attempt to summarize recent data on BVDV in the context of our previous understanding of its molecular biology. Reference will be made to the other two pestiviruses, hog cholera virus and border disease virus, where pertinent. Discussions of the prototypes of other genera of the flaviviridae, yellow fever virus, and hepatitis C virus will be avoided; readers are referred to excellent reviews in the literature.^{22, 23}

Bovine, ovine, and swine pestiviruses infect and replicate interchangeably in cells of any of these species, albeit with more or less efficiency.^{15, 51, 74, 93, 126} Infection typically does not result in morphologic changes in the infected cell, thus these viruses are called noncytopathic (NCP). NCP-BVDV virus accounts for more than 90% of infections in cattle, thus we consider this biotype to be the standard BVDV.⁴⁷ BVDV frequently is isolated from fatal cases of mucosal disease.^{19, 24, 61, 86, 121, 129} BVDV isolated from these cases often causes cytopathic (CP) effects in infected cultured cells and is called CP-BVDV.^{56, 78} CP-BVDV isolates are an eclectic group in nature with a variety of genomic abnormalities.^{80-82, 105, 118, 119} We should note that although the standard BVDV is a noncytopathic virus, most in vitro and biochemical analyses discussed in this review were performed using one of two strains of CP-BVDV: NADL or Singer strains.^{25, 27}

A PORTRAIT OF BVDV

The Genomic RNA Molecule: Structure

Genetic information of standard BVDV is stored in a single molecule of RNA. Early studies showed that naked genomic RNA extracted from virions, if transfected into bovine cells, gives rise to infectious viral progeny.³⁶ No RNA molecules of subgenomic size are found in virus preparations or infected cells.¹⁰⁴ Eukaryotic cells lack enzymes to replicate RNA molecules using RNA templates; it follows that transfected BVDV RNA must program translation of viral enzymes with RNA replicase function.

Sequence analysis of cDNA cloned into plasmid vectors has shown that the standard genome of BVDV is 12,308 nucleotides (nt) in length (CP-BVDV are an exception).^{26, 34, 80, 105, 107} The base composition is 32%A, 22%T, 26%G, and 20%C, a slightly AT-rich distribution.³⁴ A single large open reading frame (ORF) is found starting at nt 386 of the plus sense of the RNA.³⁴ This ORF is 3898 codons long. Several very short ORFs (<30 codons) are found elsewhere in the plus or minus sense RNA.^{34, 35} These small ORFs apparently are not translated, thus the assertion BVDV has a single open reading frame. BVDV polyprotein, translated from the single large ORF, gives rise to mature viral proteins by viral and cellular

endoprotease cleavages. The standard BVDV genome does not have redundant sequences. There is only limited intramolecular complementarity with potential for stable secondary structure in the genomic RNA.

The ends of the RNA flanking the ORF are called the 5' and 3' untranslated regions (UTR). The BVDV genomic RNA molecule seems to lack two typical structures found at the termini of a majority of eukaryotic mRNA: 5' cap and 3' poly A structures.^{18, 26} Instead, UTRs of significant length are present: the 5'UTR is nt 385 in length, whereas the 3'UTR is nt 226 long. Considerable space to accommodate information for *cis* or *trans* functions is available. UTRs may be functional equivalents of the 5' cap and poly A, controlling translation initiation and RNA stability, respectively.^{46, 68, 88, 97, 133} Both ends must contain signals to guide replicase entry: the 3' end for plus sense (vRNA) and the 5' end for the minus strand (cRNA) template recognition. Purchio et al observed that RNA extracted from virions with organic solvents to free it of proteins can be used to program *in vitro* translation systems prepared from reticulocyte lysates, in agreement with previous reports about the infectious nature of viral RNA.¹⁰⁴ Experimental evidence consistent with the notion that BVDV lacks an inverted methyl guanosine, a cap structure, at the 5' end of its genomic RNA has been presented.¹⁸ Attempts to retain RNA in a chromatography matrix containing oligo (U) failed, indicating that the virus lacked a polyA stretch at the 3' end.²⁶ These findings were extended by Deng and Brock, who reported the sequence of the termini of BVDV. The 3' end of the vRNA consists of a stretch of 5 cytidines (C₅).^{18, 35} The 3' end of the pestivirus genome seems to be unique among flaviviridae in lacking a homopolymeric tail.⁶³ This homopolymer is usually polyA except for human hepatitis C virus, which may have poly U.⁶³ The arthropod-borne members of flaviviridae have 5' capped RNA genomes, unlike BVDV and hepatitis C virus (Table 1).⁵⁵

Sequence Comparisons Among Pestiviruses

Pairwise nucleotide sequence comparisons among different BVDV isolates show an overall conservation of the sequence. The most divergent isolates differ by 22% across the entire genome.^{25-27, 34} It is likely that sequencing of more divergent isolates will show differences of approximately 30%. Sequence conservation is not uniform along the genome. The most conserved nucleotide sequence blocks can be found in the 5'UTR (Figs. 1 and 2). Least conserved sequences are insertions of host cell-derived genetic information into p125/NS23 of some isolates of cytopathic BVDV (Figs. 1 and 3). Excepting these anomalies found in altered BVDV genomes, four stretches of very low sequence conservation are found among standard BVDV genomes.³⁴ Two hypervariable stretches are found in a region encoding for the major surface glycoprotein, gp53/E2. Two others are located in the nonstructural polypeptides p125/NS23 and p58/NS5A (see Fig. 1). Only one sizable deletion has been described in a replication-competent virus. The genome of the

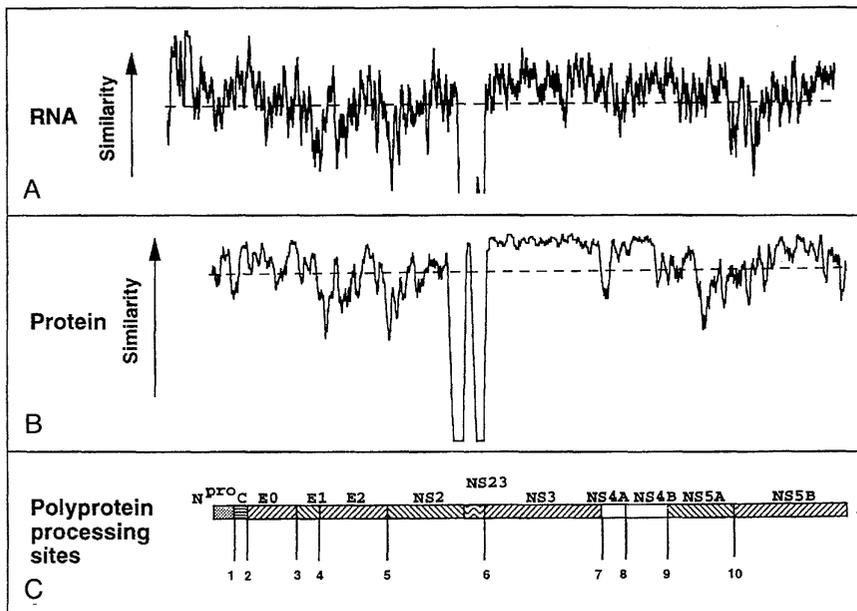


Figure 1. Similarity plots of pestivirus genomes. BVDV strains NADL, Osloss, and SD1 were compared with Hog cholera strains Brescia and Alfort over the entire length of their genomes (A) or the whole polyprotein (B). The viral proteins encoded in each region and the protease cleavage sites, numbered 1 to 10 are shown in C.

Osloss strain has a deletion of nt 50 in the 3'UTR.³³ It is not clear if these deletions are present in field isolates or represent in vitro selection events.

Ridpath et al have shown that two distinct genetic lineages of BVDV can be identified.^{96, 108} Laboratory strains of BVDV whose genomes have been sequenced, such as NADL, Osloss (CP) and SD-1 (NCP), belong to one of these lineages.^{96, 108} Comparison of the nucleotide sequences among these classical strains shows a sequence homology of nearly 78% to 88% for the entire genome. Conserved regions such as the 5'UTR of these viruses have a homology ranging from 86% to 93%. Newly described BVDV isolates have 5'-UTR sequences only 75% homologous to the classical strains, and more than 90% homologous when compared with each other. The newly identified lineage is termed *genotype II*; traditional or classical isolates are termed *genotype I*.^{96, 108} Genetic dis-

Figure 2. Computer-predicted secondary structure diagram of the 5'UTR of BVDV genomic RNA (from nucleotide 1 to 385). Letters refer to domains as described elsewhere.³⁵ Alignments of highly conserved sequences of hog cholera virus, BVDV, and human hepatitis C virus are shown in boxes. Shading highlights complete conservation in the structure and the alignments.

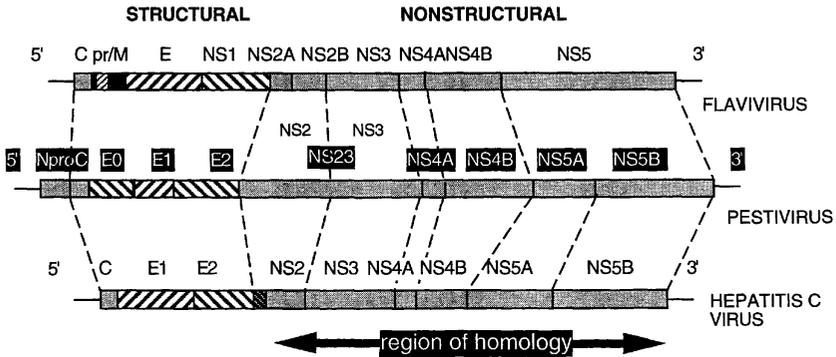


Figure 3. Comparative genomic organization BVDV (pestivirus prototype), Yellow fever (flavivirus), and hepatitis C virus (genus to be named) based on the amino acid sequence homology of the nonstructural regions and the functions of the structural genes.

tances are correlated with the extensive antigenic differences between virion surface glycoprotein gp53/E2 of genotype I and II viruses.^{96, 108}

Nucleotide sequences of the entire genomic RNA of BVDV, hog cholera virus, and part of border disease virus are available for comparison.^{5, 26, 34, 79, 89, 115} The overall nucleotide sequence similarity between BVDV and swine pestivirus genomes oscillates around 67%. Differences are unevenly distributed: the 5'UTR is highly conserved, whereas the remainder of the genome is more divergent (see Figs. 1 and 2). The overall profile of sequence similarity among pestiviruses is very similar to the sequence comparison among BVDV isolates. Distinct lineages of hog cholera virus, analogous to the genotypes I and II of BVDV, have not been reported.

Sequence Comparison of Pestiviruses With Other Flaviviruses

Nucleotide sequence comparisons between the BVDV genome and other viruses were first made by Collett and Renard showing that pestiviruses share statistically significant sequence similarities to the flaviviruses.^{26, 107} This similarity, however, is restricted to a few 20- to 30-nucleotide blocks with 80% identity matching, dispersed in different areas of the genome. Nucleotide sequence similarity between BVDV and viruses in the other two genera of flaviviridae shows that hepatitis C virus has a most striking homology in the 5'UTR (see Fig. 2).^{21, 23, 83}

Nucleotide sequence identity levels in other regions of the genome are marginal, and statistical analyses are required to demonstrate that they are not just random matches. The conservation of the 5'UTR across highly divergent genera of the family flaviviridae points to some fundamental role of importance for viral replication. Recent experiments with hepatitis C virus suggest that this region of the genome can direct

ribosomes to initiate translation from a downstream AUG triplet by a cap-independent mechanism.¹²²

Wobble and redundancy in the genetic code make comparison of nucleotide sequence similarity of protein-coding regions less sensitive to detect distant relationships than the alignment of the amino acid sequence of the encoded protein. Comparison of the encoded proteins sequences will often show significant similarities when nucleotide comparisons failed to do so. Alignment of pestiviral polyproteins with polyprotein of viruses in the other genera of flaviviridae shows a pattern of conserved blocks of sequence homology spread along most of the nonstructural protein region (Fig. 3).

The Proteins Encoded by BVDV

The standard genome of the virus has a large open reading frame with capacity to encode a polyprotein of 3898 amino acids and, theoretically, a 438 kD protein.³⁴ With the exception of a fraction of cytopathic isolates, this sequence is nonredundant. Occasional sequence redundancy and variations in the size of the ORF result from the presence of rearranged and duplicated viral sequences or insertions of host cell RNA into the viral genome of cytopathic isolates (Fig. 4).^{60, 80-82, 105, 118, 119}

Analysis of the chemical properties of the amino acids along the polyprotein chain shows regions with substantial clustering of hydrophobic and hydrophilic residues. The first 250 amino acids at the N-terminus of the polyprotein are predominantly hydrophilic. A very hydrophobic stretch of 266 amino acids is located at the N-terminus of the nonstructural protein region, between codons 1036 and 1301. The BVDV polyprotein has 14 potential glycosylation sites in the region encoding for structural polypeptides. Eleven glycosylation consensus sites are conserved among BVDV isolates, and 8 of these are present in swine and bovine pestiviruses.

The BVDV genome, like that of all positive strand RNA viruses, encodes for structural and nonstructural proteins whose role is to make

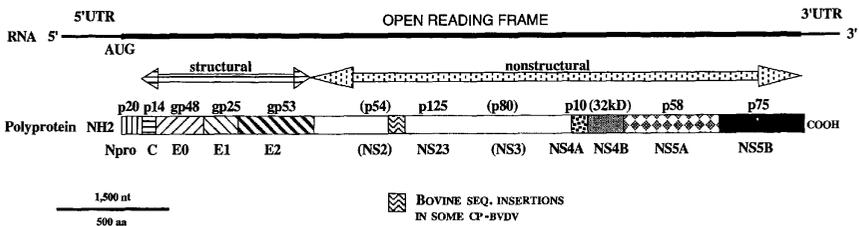


Figure 4. Protein map of BVDV. Note a polypeptide at the N-terminus of the polyprotein, the leader protease p20/Npro does not serve a structural role. Polypeptides in parenthesis are not present in all virus isolates. The 32 kD polypeptide has not been identified unambiguously.

up virions and replicate the genome, respectively. Viral proteins from two sources have been studied: purified virions and infected cell lysates. For biochemical analysis of structural proteins from virions, infected cells are grown in a culture medium supplemented with a radioactive isotope-containing amino acid. Metabolically radiolabeled virus is harvested from the medium and purified by density gradient centrifugation for subsequent analysis by denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of nonstructural viral polypeptides in cells can also be performed by metabolic labeling of infected cells. Unlike many other viral infections, BVDV infection does not inhibit cell macromolecular synthesis. Inhibition of labeled amino acid incorporation into cellular polypeptides is thus required to identify viral polypeptides after electrophoresis, e.g., using hypertonic medium.^{42, 44} Alternatively, viral polypeptides can be distinguished from cellular protein by binding of specific antiviral antibodies, e.g., radioimmunoprecipitation and western blotting.^{43, 45, 54} These methods obviously are limited by the specificity of the polyclonal antiserum or the availability of a monoclonal antibody.

A total of at least 13 virus-induced polypeptides (structural and nonstructural) have been found in BVDV infected cells by biochemical analysis to identify viral proteins.^{2, 3, 25-27, 42, 44} The sum of the molecular weights of these polypeptides (>550 kD) exceeds the coding capacity of the genome (437 kD) and outweighs the sum of predicted mature products of the polyprotein after glycosylation of potential sites (465 kD). Sequence redundancy among the 13 polypeptides identified by SDS-PAGE analysis of BVDV infected cells was predicated by biochemical and immunologic means.¹ Peptide mapping of two polypeptides of 80 and 125 kD showed a number of shared peptides in the fingerprint, consistent with the presence of regions of identical amino acid sequences.¹ Availability of monoclonal antibodies to some of these proteins subsequently showed that a single antibody recognized two or more polypeptides of different sizes, corroborating peptide mapping results.^{8, 29, 85, 109}

Molecular cloning of the BVDV genome and determination of the primary structure of the genomic RNA paved the way to the establishment of the first genetic map of BVDV. Alignment of deduced BVDV protein sequences with other flaviviruses, reactivity of viral proteins with monoclonal antibodies and region- or peptide-specific antisera led to the initial protein map of BVDV.²⁶ The latter were produced from short segments of the BVDV genome cloned into bacterial expression vectors. Purified BVDV- β -galactosidase fusion proteins expressed in recombinant bacteria were injected into rabbits to increase region-specific antibodies. Viral proteins in BVDV-infected bovine cells were identified with these antibodies by radioimmunoprecipitation. This approach led to a map of the genomic regions encoding most of the viral polypeptides (Fig. 4).^{25, 27} Further refinement of the map was achieved with the use of rabbit antibodies against synthetic peptides deduced from the genomic nucleotide sequence.² The resulting map indicated very clearly that the BVDV polyprotein resulted in several processing intermediates before the mature viral polypeptides were produced. Previous difficulties to

interpret existing data were virtually eliminated. Some of the proteolytic processing events were proposed to be cotranslational, because the entire 438 kD polyprotein was never identified in infected cells. Despite remarkable progress made in viral protein mapping and identification, the protein map of BVDV remains to be refined. Identification of a nonstructural polypeptide from a region of the genome that is highly conserved (NS4) awaits future experimental efforts. Precise determination of cleavage sites within the nonstructural region needs to be completed.

The current protein map of the BVDV genome is shown in Figure 4. Pestiviral polypeptide nomenclature is still being elaborated. Initial use of polypeptide molecular weights was necessary because the role of the proteins was unknown. With current information, a more rational system will be developed to allow discussion of all the pestiviruses and other members of flaviviridae under a common system. Until then, we chose to use both nomenclatures in the text to facilitate the transition (see Fig. 4). A total of at least 10 mature viral polypeptides are produced from the entire polyprotein of standard NCP-BVDV. Four of these polypeptides are structural, and six are nonstructural. CP-BVDV isolates express one or more additional nonstructural polypeptide(s).^{25, 27}

The N-terminal end of standard BVDV polyprotein results in a nonstructural protein p20/Npro (Npro, N-terminal protease). The adjacent region of the polyprotein yields the polypeptide chains for the four putative viral structural polypeptides: p14/C (C, nucleocapsid), gp48, envelope 0 (E0), gp25/E1 (envelope 1), and gp53/E2 (envelope 2). The remainder of the polyprotein encodes for five nonstructural proteins in N to C order: p125/NS23, p10/NS4A, p32/NS4B, p58/NS5A and p75/NS5B. The identity of p32/NS4B is still speculative. In addition to these proteins expressed by standard BVDV, CP-BVDV isolates invariably express p80/NS3 and, occasionally, other polypeptides of variable size. Variable amounts of additional polypeptides of sizes other than those shown in Figure 4 can often be identified in infected cells and may represent processing at alternative sites.² Their significance in virus replication, as well as that of incompletely processed polypeptides, is still unknown.

The following section will describe the main features of the mature gene products of standard BVDV. For simplicity, discussion will follow the sequence in which they are arranged in the genome.

p20/Npro

p20/Npro is the first translation product of the open reading frame. This protein consists of 168 amino acids, with an experimentally observed mass of 20 kD in SDS-PAGE. p20 is a *cis*-acting papain-like protease that cleaves intramolecularly at its C-terminus, effectively releasing itself from the nascent polyprotein. The protease recognition sequence for cleavage is Cysteine-Serine, with cleavage after cysteine. Functions for this papain-like protease in virus replication other than the generation of the N-terminus of p14/C have not been identified.

Absence of a counterpart in other flaviviruses precludes comparative analyses. Similar accessory leader proteases are present in foot and mouth disease virus, equine arteritis virus, and a plant potyvirus.^{59, 70, 73} The leader protease of foot and mouth disease virus is implicated in host cell translation shut-off, but BVDV infection does not induce shut-off of host cell translation.¹²⁰

p14/C

This protein has 102 amino acids and migrates as a 14 kD polypeptide. *p14/C* is a basic protein with an isoelectric point of 10.69 (SD-1 strain) due to a 26% molar percentage of basic amino acid residues. The protein is well conserved across different pestiviruses. Its N-terminus is generated by the autocatalytic action of *p20/Npro* and seems to be blocked by posttranslational modification. *p14/C* presumably is located in the cytoplasm of infected cells. It is not known whether it migrates to other compartments. The function of the protein is to package the genomic RNA and to provide necessary interactions for formation of the enveloped virion. Domains involved in these processes have not been identified. Sera from convalescent cattle do not contain antibodies to *p14/C*.⁴⁵ The poor immunogenicity of the BVDV capsid in cattle contrasts with the abundance of antibody to the hepatitis C capsid protein in infected human sera.⁶⁹

gp48/E0rns

This glycoprotein has 227 amino acids (mature form) with a predicted mass of 26 kD and observed migration on SDS PAGE of 48kD. Carbohydrate moieties attached to the seven to eight consensus glycosylation sites are responsible for the differences. A signal sequence for translocation into the endoplasmic reticulum (ER) is present at the N-terminus. A hydrophobic anchor region is lacking throughout the sequence of the polypeptide. Signalase cleavage in the ER lumen results in a very hydrophilic mature *gp48/E0*. It forms homodimers covalently linked by disulfides, after its translocation to the lumen of the ER. The function of *gp48/E0* is unclear; it is a putative component of the virion, although the absence of a hydrophobic membrane anchor region suggests a loose interaction with the envelope. The structure of the oligosaccharides attached to *gp48/E0* has not been determined. It is unknown if the protein has covalently attached fatty acids. *gp48/E0* appears to be secreted to the extracellular space by exocytosis. Comparison of the BVDV *gp48/E0* with other pestiviruses shows remarkable conservation. RNase activity has been detected in purified preparations of hog cholera virus *gp46/E0*; a similar activity is predicted for the BVDV counterpart, *gp48*.¹¹⁴ The hog cholera virus and BVDV protein sequences contain two conserved motifs similar to fungal and plant RNase domains.^{67, 114} The significance of this enzymatic activity is unknown, yet very intriguing because of the sequence conservation. This glycoprotein induces consid-

erable levels of antibodies in infected cattle, but these antibodies have limited virus-neutralizing activity.^{17, 131}

gp25/E1

This polypeptide is predicted to be 195 amino acids in length, with two consensus sites for glycosylation. A calculated molecular weight of 21.6 kD is smaller than the experimentally observed 25 kD because of posttranslational modification. The protein contains two hydrophobic domains that serve to anchor the protein in the membrane and initiate translocation of the adjacent polypeptide, gp53/E2. gp25/E1 is found in virions covalently linked to gp53/E2 by disulfide bonds. Convalescent cattle serum does not contain significant levels of antibody to gp25/E1.⁴⁵

gp53/E2

The polypeptide backbone of gp53/E2 is approximately 375 to 400 amino acids long and contains three to four consensus sites for glycosylation. The N-terminal end is generated by signalase cleavage, predicted with very good reliability. The uncertainty surrounding its size is derived from lack of information on the C-terminal processing site(s), also presumed to be cleaved by signalase. There is preliminary evidence indicating that two different forms of gp53/E2 can be found in infected cells; the difference is found in their C-terminal tail length. The predicted mass of the polypeptide is 41 to 45 kD, but postranslational modification results in apparent migration of 53 ± 2 kD. The glycoprotein is likely to be found in the virion envelope as homodimers and as heterodimers with gp25. The C-terminus of gp53/E2 is anchored in the lipid envelope by a transmembrane region of at least 40 amino acids, which may span the membrane more than once. gp53/E2 is very antigenic and elicits the production of neutralizing antibodies in the host after infection or vaccination with live or killed vaccines. One of the three hypervariable sequence regions found in the viral genome is present in this polypeptide. This hypervariability may represent absence of structural constraints and perhaps reflects immunologic selective pressure.^{9, 10, 13, 40, 45}

p125/NS23

This BVDV nonstructural protein has approximately 1300 amino acids and is slightly basic, with a pI of 8. It migrates in SDS-PAGE gels as a 125 kD polypeptide. Sequences of the N- and C-termini have not been determined. Sequence similarity searches show that p125/NS23 has homology to other flaviviruses: p125/NS23 seems to encompass a region that in flaviviridae is divided into two separate polypeptides, NS2 and NS3. This domain separation in p125/NS23 correlates with distinct chemical properties for each of them. The N-terminal region, homologous to NS2 is very hydrophobic. The C-terminal domain is hydrophilic and homologous to NS3. The p125/NS23 polypeptide, and

its C-terminal colinear fragment, p80/NS3, are the most studied proteins of BVDV. p80/NS3 has received a great deal of attention because it is found exclusively in cytopathic BVDV isolates.^{43, 100} Cytopathic isolates of BVDV produce p80/NS3 as a distinct polypeptide. The expression of p80/NS3 as a separate polypeptide resembles the protein profile of the arthropod-borne flaviviruses.

Four important domains are present in this large polypeptide: A very hydrophobic domain at the N-terminus, followed by a zinc finger, a protease, and a helicase, towards the C-terminus.³⁴ A cysteine-rich domain found between codons 1484 and 1512 (approximately 384 codons from the p125/NS23 N-terminus) was predicted to form a zinc finger.³² This domain is thought to bind RNA. An RNA-binding domain may serve to hold the viral replication complex in close proximity to the membrane through the N-terminal hydrophobic domain. A region of homology between BVDV and a number of plant and animal viral chymotrypsin-like serine proteases is found between codons 1658 and 1752 (approximately 568 codons from the N-terminus).⁴ Mutagenic analysis has shown that this site encompasses the catalytic triad of the viral protease responsible for the cleavage of the polyprotein to generate nonstructural polypeptides by a series of intramolecular and intermolecular cleavages.¹³⁰ A domain with homology to eukaryotic helicases has been identified between codons 1817 and 2095 (starting at 333 amino acids from the N-terminus of p125/NS23).^{57, 58} This domain binds ATP and hydrolyzes it in an RNA-stimulated manner.¹¹⁷ This helicase is able to catalyze ATP-dependent strand separation of RNA duplexes.¹²⁵ A role for the helicase in virus replication has not been found. Helicase participation is postulated for translation initiation and RNA replication.

Insertions of host cell genetic elements were found in the p125/NS23 of some cytopathic isolates of CP-BVDV. Other CP-BVDV isolates have rearrangements of viral sequences, with duplication of genome sequences in the p125/NS23 region. All of these result in *de novo* expression of p80/NS3, sometimes altering the size of p125/NS23. Whatever the mechanistic paths of p80/NS3 expression, p125/NS23 expression is always maintained.^{25, 47, 60, 80-82, 117, 118} Thus, p125/NS23 must play an indispensable role in virus replication. The protease and helicase domains of p125/NS23 are present in the region that is colinear with p80/NS3. Cytopathic isolates of BVDV invariably express p80/NS3, suggesting that this protein is directly or indirectly involved in cellular damage. The possible role of protease and helicase enzymatic activities in cytopathology is the subject of intense research.

Infected cattle (and cattle vaccinated with modified live BVDV vaccine) develop a strong humoral antibody response to p125/NS23. Cattle vaccinated with killed vaccine develop negligible humoral antibody to this nonstructural polypeptide. Antibodies to BVDV p125/NS23 cross-react with hog cholera virus p125/NS23 and vice versa; swine antibodies to hog cholera p125/NS23 cross-react with BVDV.^{94, 127, 128} A similar situation exists with border disease virus regarding immune crossreactivity.

(p54/NS2)

This nonstructural polypeptide is found exclusively in some, but not all cytopathic isolates of BVDV. When present its size is highly variable because of the existence of additional genetic elements due to BVDV sequence rearrangement or foreign sequence insertions. p54 is a proteolytic product of p125/NS23, colinear with its N-terminus. This polypeptide has a zinc finger motif and is hydrophobic. This portion of p125/NS23 is poorly immunogenic and does not induce humoral antibodies in infected cattle.^{13, 45}

(p80/NS3)

This hydrophilic nonstructural polypeptide is a marker of cytopathic BVDV. Its sequence is colinear with the C-terminus of p125/NS23, and contains the protease and helicase domains. p80/NS3 is homologous to the flaviviral NS3. Both termini of p80/NS3 are generated by autoproteolytic cleavage in *cis*. Functional constraints must be limiting the evolution of this polypeptide severely; p80/NS3 is the most conserved protein in the genus *Pestivirus*. This polypeptide is very stable in infected cells and highly immunogenic.^{13, 45}

p10/NS4A

Virtually no information is available on this polypeptide. Boundaries (processing sites) are defined imprecisely. Its sequence is well conserved among different pestiviruses, suggesting possible functional constraints and an important role in replication.

p32/NS4B

Little is known about p32/NS4B; the precise location of processing sites and its function remain elusive. Predictions based on sequence analyses would suggest that this is a relatively basic polypeptide with a pI of approximately 9.6. Wiskerkerchen reported a possible role for p32 in modulating the protease activity of p125/NS23 in the generation of p75/NS5B by cleavage at site 10 (see below).¹³⁰ The high level of sequence conservation suggests some as yet unknown important function for this polypeptide. Preliminary data suggest that this polypeptide accumulates in infected cells at late stages of infection with CP-BVDV. Cattle infected with BVDV fail to produce humoral antibodies to p32.

p58/NS5A

Knowledge about this protein is limited. Its boundaries within the polyprotein have not been defined. Blocks of homology between this protein and the N-terminus of the NS5 (flavivirus RNA replicase polypeptide) suggest some role in RNA synthesis. Pestiviruses seem to have

split its RNA polymerase activity in two polypeptides, p58 NS5A and p75/NS5B, in contrast with the arthropod borne flaviviruses, which have just one polypeptide, NS5. An alternative active form of the replicase may be a precursor polypeptide, p133, which is cleaved to yield p58/NS5A and p75/NS5B. Hepatitis C virus is similar to pestiviruses; two polypeptides are produced by cleavage of the polyprotein in this region, the main replicase and a possible accessory polypeptide. This polypeptide is relatively stable in infected cells. The sequence of this polypeptide displays considerable variability among pestiviruses. No humoral immune response to this polypeptide can be detected in convalescent cattle.^{13, 45}

p75/NS5B

This polypeptide has yet to be fully characterized biochemically and the processing site to be identified. p75/NS5B is the putative viral RNA-dependent-RNA polymerase. The canonical motif present in all positive strand viral RNA polymerases, glycine-aspartate-aspartate, can be found between codons 3626 and 3628. The half-life of p75/NS5B is among the shortest of all BVDV polypeptides. No serum antibodies to p75/NS5B are found in cattle recovered from BVDV infection.^{13, 45}

Structure of the Virion: the RNA Delivery Apparatus

The BVDV virion consists of a central core containing RNA and the capsid protein p14/C, surrounded by a lipid membrane with two glycoproteins anchored in it, gp25/E1 and gp53/E2.^{25, 27} A third glycoprotein (gp48/E0) is found in virions, loosely associated to the envelope by uncharacterized interactions. The infectious nature of the naked RNA indicates that there are no proteins required in virions to catalyze RNA replication. The function of the proteins and lipids surrounding the RNA molecule is to "pick up" the viral genome in the cytosol of infected cells and to deliver it to the cytosol of an uninfected target cell. Structural proteins are required to perform two seemingly opposing tasks. These are (1) to assemble virions wrapping the RNA and exit the infected cell, and (2) to enter the target cell and disassemble to release the RNA. The target cell may be a few microns away or in another host. The versatility of the virion must be considerable.

Virions are 40 to 60 nm in diameter, with a sedimentation coefficient $S_{20w} = 140$ and a buoyant density of 1.12 to 1.13 in sucrose gradients (see Table 1).^{30, 64, 66, 72} The core is composed of a single RNA molecule covered by the nucleocapsid protein p14/C. Conclusive determination of the composition of virion envelopes remains elusive. Three glycoproteins seem to be associated with the virion envelope at different stages of maturation: gp48/E0, gp25/E1, and gp53/E2. Electrophoretic migration of the glycoproteins in SDS-PAGE is quite diffuse because of the charge and trimming heterogeneity of their oligosaccharide chains.⁴⁴ The struc-

ture of the N-linked oligosaccharide chains of the glycoproteins has not been determined. It is not known if there are additional O-linked sugars attached to the glycoproteins.

Taxonomy of BVDV

The Genus Pestivirus: Shared Features

Members of the genus are the bovine, swine, and ovine pestiviruses. Members of the genus *Pestivirus* are closely related to each other. *Pestivirus* virions are morphologically and physicochemically similar.^{30, 55, 65, 71, 72} The genomic RNA is virtually the same size, and the structural and nonstructural polypeptides are very similar. Pestiviruses display mutual antigenic cross-reactivity.^{31, 98} Members of the genus have restricted host range in vivo and in vitro.^{15, 93} No insect vectors have been identified for members of the genus *Pestivirus*. The replication cycle in cells is virtually identical for members of the genus, and the strategy of gene expression has few differences.

Pestivirus identification is often problematic because their close relatedness makes assignment to a host species (ruminant or swine) extremely difficult. The serologic cross-reactivity between hog cholera virus and bovine viral diarrhea was discovered shortly after the virus was isolated in cell cultures.^{31, 98} Cross-reactivity is more apparent with antibodies to nonstructural proteins.⁹⁴ The level of neutralization of infectivity of heterologous virus by polyclonal antiserum from convalescent animals is very low. Swine pestiviruses can infect cattle, and cattle pestiviruses can infect pigs, both inducing cross-reactive immune responses. The diagnostic conundrum that this situation creates is a challenge for diagnostic virologists and serologists, in parts of the world in which both viruses are present.^{127, 128}

Family Flaviviridae: Distant Relationships

The family flaviviridae comprises a diverse group of viruses grouped in three genera: *Flavivirus*, (formerly Arbovirus group B), *Pestivirus* (previously a nonarbo togavirus, mucosal disease group), and a recently discovered human virus hepatitis C virus (a genus name has not been assigned yet) (see Table 1). The common ancestry of these viruses is evidenced by the conservation of blocks of amino acid sequences in the nonstructural proteins of viruses of the family.^{27, 70} The extent of conservation of these motifs is greater comparing members of the family to each other than when comparing them to other positive stranded RNA viruses.^{55, 63} Conservation of blocks of amino acid sequence similarity across most of the nonstructural region of the genome points to the common ancestry of these viruses. Phylogenetic analysis of positive strand RNA viruses based on replicase, chymotrypsin-like protease, or the helicase sequences have all resulted in arthropod borne

Table 1. COMPARATIVE VIRION FEATURES IN FLAVIVIRIDAE

Features/Genus	Pestivirus	Hepatitis C Virus Group	Flavivirus
Virion morphology	40–60 nm	40–60 nm(?)	40–60 nm
Sedimentation	S20w = 140	S20w = 150	S20w = 170–210
Virion density	1.12–1.13	1.09–1.11	1.15–1.200
RNA genome	12 kb	10 kb	11 kb
5' structure RNA	No 5' cap	5' cap(?)	5' capped
5'UTR	385 nt	324–341 nt	~150 nt
ORF	3898 codons	~3,000 codons	~2,800 codons
3' structure RNA	No poly A	No poly A = Oligo U (?)	No poly A
Envelope	3 gp	2 gp ?	1 gp (1 p)
Morphogenesis	ER	?	ER

flaviviruses and pestiviruses originating from a common ancestor.⁷⁰ With the noteworthy exception of the leader papain-like protease present exclusively in pestiviruses, the rest of the viral polypeptides have a similar distribution along the genome (see Fig. 3). Important divergent features in the nonstructural region are represented by processing differences: pestiviruses do not process a site that in flaviviruses separates NS2 from NS3. In contrast, pestiviruses process NS5 into NS5A and B.^{22, 25, 26} Besides the obvious conservation of critical motifs in replicase and protease-helicase, striking similarities between BVDV and hepatitis C virus are found in the 5'UTR.^{23, 35, 63, 83}

The *in vivo* host range of a typical flavivirus includes mammals as well as insects. The host range of pestiviruses and human hepatitis C virus is restricted to a cluster of closely related mammalian species, i.e., Artiodactyla and Primate, respectively. A similar picture emerges for the ability of these viruses to grow *in vitro*: members of the genus *Flavivirus* grow in insect and various mammalian and avian cells; Pestiviruses are restricted to growth in cell cultures of Artiodactyla, whereas hepatitis C does not replicate efficiently in any cell culture.⁵⁵

A MOTION PICTURE OF BVDV

The Replication of BVDV

BVDV replicates efficiently only in cells derived from Artiodactyla, closely reflecting the *in vivo* host range of the virus.¹⁵ Optimal replication takes place in bovine, ovine, or goat cells, whereas replication is less efficient in swine cells.¹⁵ BVDV can be adapted with difficulty to replicate in rabbit cells. Rabbits are susceptible to infection with rabbit-adapted BVDV, and are the only known laboratory animal host for the virus.⁵² The replication of standard BVDV and other pestiviruses in cell cultures of bovine origin takes place without production of visible changes in the cells.⁸⁷ In some cases, these viruses contaminate cell cultures for years without detectable alterations of cell physiology or metabolism.^{11, 53, 77, 90, 103, 113}

When BVDV comes in contact with a susceptible host cell, it initiates a series of steps that result in the production of progeny virions. This is termed a *productive replication cycle* and takes place entirely in the cytoplasm of the cell. The peak of progeny virus release occurs 12 to 14 hours after start of infection.⁸⁴ Each infected cell releases 100 to 1000 infectious virions (Donis RO, unpublished data). The BVDV replication cycle was divided into phases, with somewhat arbitrary boundaries, for the sake of discussion in the next sections.

Attachment and Penetration

BVDV enters cells by receptor-mediated endocytosis, followed by an acid-dependent step that delivers the viral genome to the cytosol.¹⁶ Reports on the cellular and viral molecules responsible for the initial interaction and subsequent triggering of endocytosis suggest that gp53/E2 interacts with a cellular receptor of 50 kD.¹³² Integrity of cellular plasma membrane proteins is necessary for infectivity, because treatment of the cell surface with proteases renders the cells resistant to infection.⁵⁴ A mutant bovine cell line that is resistant to BVDV infection has been described.⁵⁴ The mutant cells, derived from the Madin-Darby Bone Kidney (MDBK) cell line, are resistant to infection as a result of a defect in a host factor mediating virus entry. The nature of the defect is under investigation. Because this mutant cell is resistant to swine, ovine, and bovine pestiviruses, it is likely that pestiviruses use a common mechanism or pathway to enter target cells (Flores EF, Donis RO, unpublished data).

Besides the postulated interaction of gp53/E2 with cellular molecules for attachment of virions to target cells, other surface glycoproteins of BVDV may participate in this process. BVDV seems to be extremely sensitive to the binding of monoclonal antibodies to its E2, an event that often leads to neutralization.^{8, 41} This loss of infectivity is not simply consequence of binding inhibition because most of these monoclonal antibodies are capable of neutralizing virus after attachment to target cells.¹⁶ Antibodies to gp48 have low neutralizing capacity, and it is not known if they interfere with virus attachment by a mechanism other than virion aggregation.¹⁶

After the initial virion attachment, an endocytosis step is required for infection. Endocytosis blockers such as phenylarsine oxide are efficient inhibitors of virus infection.⁵⁴ Infection proceeds if endocytosis is restored by reversing the chemical block with a reducing agent. Endosome acidification is required for virus entry, as evidenced by the ability of chloroquine or ammonium hydroxide to block infection (Donis RO, unpublished data).¹⁶ If these drugs are added immediately after the virus entry step, they have no effect on virus replication. Presumably, increasing endosomal proton concentrations are required to trigger fusion of viral envelope with cellular membranes and release of the viral genome into the cytosol. Virtually nothing is known about the fusion event, conditional mutants have not been identified. Events collectively

described under attachment and penetration essentially are complete in 30 minutes.

Gene Translation

The second phase of the replication cycle starts with the release of viral RNA, in the form of a ribonucleoprotein complex into the cytosol of the cell. The complex comes in contact with ribosomes, but signals directing its intracellular trafficking are not known. Translation of the viral genome requires at least partial stripping of the capsid protein p14 off the RNA, to start the first virus-directed macromolecular biosynthetic event in the infected cell. In the absence of a cap structure at the 5' end of the viral genome, the ability of viral RNA to recruit translation initiation factors must be ascribed to some domain of the 385 nucleotide-long 5'UTR.¹⁰¹ Fine mapping of this important *cis* function awaits experimental resolution. Differences in translation initiation requirements for viral and cellular mRNAs are evidenced by the ability of hypertonic cellular environments to selectively block cellular mRNA translation without effects on viral translation initiation rates. The ability of the BVDV 5'UTR to function as internal ribosome entry site (IRES) for translation of a downstream open reading frame has been demonstrated experimentally in BHK cells.¹⁰¹ Viral polypeptides can be detected in infected cells as early as 3 hours after infection, reaching a peak at 12 to 14 hours postinfection.⁴²

Polyprotein Processing

Partially processed intermediate viral polypeptides traditionally were identified by pulse-labeling infected cells and chasing the pulsed cells for increasing periods of time before harvesting and SDS-PAGE analysis. Chasing usually shows transfer of label from the parental polypeptide to its cleavage products. Using this system, precursor-product relationships among some of the nonstructural proteins of the virus were demonstrated.¹

Experimental data to support the current processing pattern of BVDV polyprotein was derived from radioimmunoprecipitation with monoclonal antibodies and with region-specific antisera. Subsequent refinements resulted from analysis of the BVDV polyprotein expressed in eukaryotic cells by plasmid vectors often carrying bacteriophage T7 RNA polymerase promoters or infection with recombinant vaccinia virus.^{2, 26, 27}

The identity of the protease(s) involved in processing of the BVDV polyprotein began to emerge with the data presented by Purchio and Collett, showing that production of mature envelope glycoproteins requires the participation of the endoplasmic reticulum, implicating cellular signalase in the process.¹⁰⁴ Existence of a viral protease in BVDV was predicted accurately from sequence comparisons with other viruses,

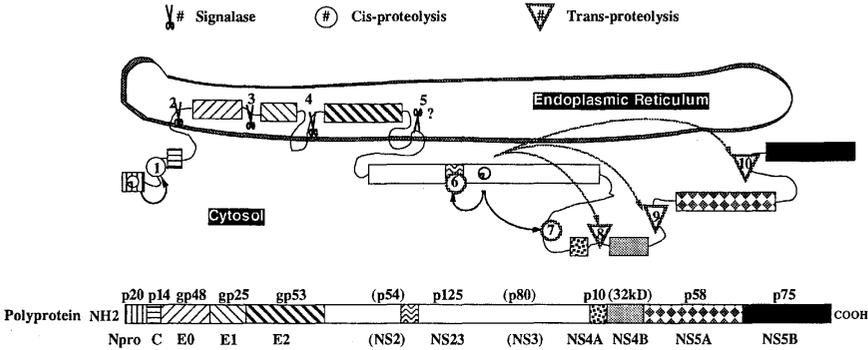


Figure 5. Polyprotein processing strategy of BVDV. The sites are numbered as in Figure 1. Site 6 is only present in some CP-BVDV isolates and is absent in NCP-BVDV.

which identified a chymotrypsin-like serine protease domain in p125/NS3.⁴

As translation of the large open reading frame to produce the viral polyprotein proceeds, viral and cellular proteases cleave the nascent polypeptides at specific sites to generate intermediates and/or mature viral proteins (Fig. 5). In this manner, nine (or 10) cleavage events generate four structural proteins and six (or seven to eight) nonstructural proteins. The variability in the number of nonstructural polypeptides, indicated parenthetically, arises from the additional polypeptide found in cytopathic BVDV. For discussion purposes, the cleavage sites have been numbered sequentially from the N- to the C-terminus of the polyprotein (see Figs. 5 and 6 for the location of the cleavage sites). Site 6 is present exclusively in some CP-BVDV; it is nonexistent in standard NCP-BVDV.

Starting from the N-terminus of the polyprotein, the first cleavage

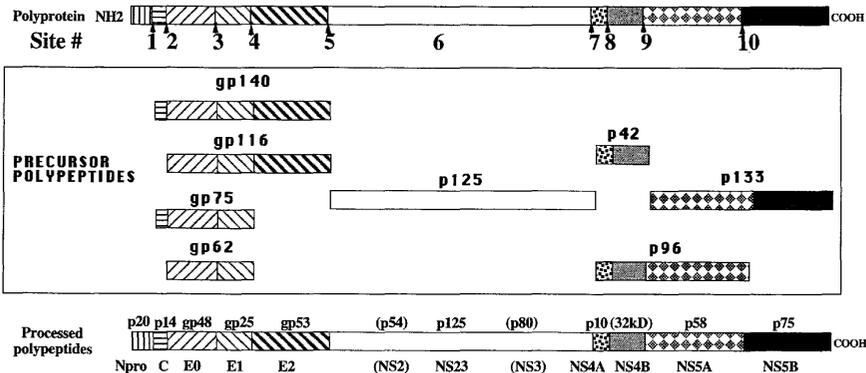


Figure 6. Map of intermediate precursor polypeptides of BVDV generated by partial cleavage of the sites mumbled in the polyprotein.

is at site 1. This site is located between the N-terminal autoprotease p20/Npro and p14/C and is catalyzed intramolecularly. Additional cleavages to generate the virion structural proteins take place in the endoplasmic reticulum or Golgi. Site 2, between p14/C and gp48/E0 and site 4 between gp25/E1 and gp53/E2 are a consequence of signalase cleavages in the lumen of the ER. These sites are cleaved rapidly after synthesis. Processing at site 3 between gp48/E0rns and gp25/E1 is delayed and seems to involve a Golgi enzyme. Cleavage at site 5 between gp53/E2 and the first nonstructural polypeptide, p125/NS23, is proposed to be effected by signalase also. The remaining cleavages to release mature viral nonstructural proteins, between p125/NS23-p10NS4A, p10/NS4A-p32/NS4B, p32/NS4B-p58/NS5A, and p58/NS5A-p75/NS5B are catalyzed by the viral protease p125/NS23.¹³⁰ Cleavage at site 7 is intramolecular and results in the production of p125/NS23 the viral protease/helicase. The rest of the cleavage sites (sites 8 to 10) are intermolecular (in trans) and give rise to the rest of the viral nonstructural proteins. Cleavage at site 10 may require p38 NS4B as a cofactor.^{25, 26} Cleavage at site 6, present exclusively in some CP-BVDV isolates such as NADL, takes place intramolecularly and results in the release of p80/NS3 and p54/NS2.¹³⁰ Several intermediate incompletely processed polypeptides can be found in infected cells at all times during infection (see Fig. 6). These polypeptides may serve additional catalytic or regulatory functions.²

Genome Replication

Once the unique viral enzymes necessary for RNA replication were produced by translation of BVDV genomic RNA and polyprotein processing, the viral life cycle enters its genome replication phase. Two well-defined processes are required to produce identical copies of the incoming plus sense genomic RNA (vRNA). First a negative strand copy is made (cRNA) to serve as template. Second, progeny vRNA is synthesized using the cRNA template from the previous step. The viral replicase and its functions have not been studied in detail.

The core of the replicase is apparently the p75/NS5B polypeptide. p75/NS5b contains an amino acid motif present in all positive strand RNA virus polymerases, containing Gly-Asp-Asp at the core.³⁴ By analogy with other positive strand RNA viruses, p75/NS5B is the candidate viral replicase. Additional polypeptides are likely to participate in RNA replication. Candidate accessory components of the replicase complex are p58/NS5A and p125/NS23. Motifs present in p58 are part of the replicase protein of yellow fever virus. p125/NS23 contains two conserved amino acid motifs: a serine protease domain and a helicase domain. Baculovirus-expressed p80/NS3 (the C-terminal fragment of p125/NS23 polypeptide) has protease activity and helicase activity.¹³¹ Helicase activity is necessary for poliovirus RNA replication.^{75, 112} Nothing is known about *cis*-acting domains (promoter-like elements) for template RNA recognition and transcription initiation by the BVDV

replicase for plus or minus strand synthesis. Regulation of plus and minus strand synthesis has not been studied.

Assembly and Budding

With the packaging of progeny RNA and assembly of virions, the cycle enters its final stages. Signals for RNA encapsidation by p14/C have not been defined. RNA binding sites on p14/C and specific structures of the viral RNA are considered to be necessary. Evidence from electron microscope studies suggests that BVDV assembly takes place in the ER or the Golgi.⁶ There are virtually no clearly visible nucleocapsid structures assembled in infected cells before extrusion into the lumen of the ER/Golgi by budding.^{111, 124} During this process, which is likely to involve contacts between p14/C and the tail of gp53/E2C, virions acquire a lipid envelope and accumulate transiently in the lumen of the vesicular compartment. Virions reach the extracellular compartment by vesicular transport (exocytosis) as early as 10 hours postinfection.

Effects of BVDV Replication on the Host Cell

Replication of standard BVDV in cell cultures *in vitro* takes place with virtually no harmful effects for the cell.⁵⁶ Attempts to ascertain subtle modifications of cellular physiology have not yielded tangible evidence. These same BVDV strains that replicate without visible/detectable effects in cell cultures can cause severe lesions that often lead to death of the host.^{7, 28} The impact of cellular differentiation state and microenvironment on the outcome of BVDV infection remains to be elucidated. Isolates of BVDV originating from cases of mucosal disease can cause severe cytopathology in cultured bovine cells.⁷⁸ The mechanisms leading to these changes are unknown. Temperature-sensitive BVDV mutants that do not cause cytopathology at the nonpermissive temperature may contribute to the understanding of this process.¹¹⁰

Evolution of BVDV

The hallmark of RNA viral genomes is their plasticity.^{38, 39} The absence of an efficient exonuclease to correct misincorporated bases results in a high frequency of base substitutions, approaching one error for every 10,000 nt polymerized. Thus the genome of an RNA virus is not a single defined entity, but an average or consensus of a heterogeneous population of molecules. The term *quasispecies* was coined to describe this concept of genomic variability.⁴⁹ Many genomes in the quasispecies will not be viable because of the lethality of certain base substitutions. RNA viruses use this strategy to generate genomes with potentially greater fitness and ability to survive under certain altered environmental conditions. The consequences on this process are seen in practice in the

form of neutralization escape mutants or the selection for viruses that are antigenically different from vaccine strains.^{40, 95}

Three major parameters have been used to characterize the viruses circulating in cattle populations: gene sequencing, antigenic analysis performed with monoclonal antibodies, and virulence studies by experimental inoculation. Recent antigenic and genetic characterization of a large number of BVDV isolates involved in atypical outbreaks of severe acute BVDV has shown the existence of much wider diversity among field virus isolates than previously acknowledged.^{96, 108} Two distinct lineages of BVDV co-circulate in cattle populations throughout the world; one is the classical genotype termed *genotype I*. The second lineage is known as *genotype II*.^{96, 108} Although these viruses have been identified recently, they seem to have been circulating in cattle populations for as long as we can analyze the viral fossil record. Genotype II BVDV prevalence, relative to genotype I, may be on the increase in the United States. There is no evidence for geographic segregation of particular lineages, consistent with the fact that cattle are the main reservoir of BVDV in nature.

Antigenic Hypervariability of Virus Glycoproteins

Nucleotide sequence analysis of several strains of BVDV has allowed the determination of genomic regions that exhibit substantial variability. These alignments indicate that two amino acid stretches within gp53/E2 glycoprotein are the most variable regions of the standard BVDV genome. Comparison of the deduced amino acid sequence indicates that a large proportion of these nucleotide substitutions result in amino acid changes in gp53/E2. gp53/E2 is the main target of the neutralizing antibody response of the host. It is tempting to speculate that the neutralizing antibody response of the host is contributing to fixation of amino acid substitutions in genomes as a result of the exclusive survival of neutralization escape gp53/E2 mutants.^{40, 95}

Highly Virulent Noncytopathic Strains of BVDV

A new paradigm of BVDV virulence was established when Rebuhn and Corapi identified noncytopathic strains of BVDV that cause severe acute disease, sometimes with severe thrombocytopenia and hemorrhagic diathesis.^{14, 28, 106} Severe acute disease is caused by certain isolates that belong to genotype II lineage.¹⁰⁸ Previous dogma established that mucosal disease was the only common pathogenetic mechanism leading to severe or fatal disease. Virulence in BVDV was associated exclusively with the ability of mucosal disease isolates to induce cytopathology. Identification of molecular markers of virulence in genotype II BVDV strains causing acute, severe, hemorrhagic disease will be of great interest. Highly virulent strains of hog cholera virus cause a disease that

resembles severe acute BVDV.^{76, 99, 116} The molecular basis of virulence in hog cholera virus infections remains to be determined.

Molecular Basis of Virulence in BVDV

Mucosal disease is the fatal sequel to fetal infection, tolerance, and virus persistence. Experimentally, CP-BVDV inoculation into persistently infected cattle reproduces the disease. Production of nonstructural polypeptide p80/NS3 was identified as a biochemical marker of CP-BVDV isolates. It also represents a marker of virulence because CP-BVDV isolates are responsible for induction of mucosal disease. The mechanistic relationships between p80/NS3, cytopathology, and mucosal disease remain to be elucidated. The main obstacle to understanding these relationships is the inability to experimentally manipulate p80/NS3 production and the ability of BVDV to induce cytopathology. The availability of a cDNA copy of the genome that yields infectious transcripts will facilitate greatly experimental approaches to understand these processes.¹²³

Biotype Diversity: Cytopathic Strains In Vitro and In Vivo

In Vitro: Expression of p80/NS3. Multiple pathways lead to p80/NS3 expression by cytopathic BVDV.⁴⁷ These include recombination or base substitutions in the genomic RNA (Fig. 7).^{47, 60, 80–82, 105} Incorporation of foreign sequences into the BVDV genome is a common finding in CP-BVDV isolates. The foreign sequence insert in the Osloss strain encodes for exactly one bovine ubiquitin monomer, whereas the insert in NADL hybridized to uncharacterized bovine sequences (see Fig. 7). The insertion of cellular sequences in certain CP-BVDV isolates involves additional rearrangement and duplication of viral sequences. Whatever the changes, the result is a newly acquired p125/NS23 processing function to yield p80/NS3 or creation of a duplicated minigene expressing p80/NS3.^{47, 60, 80–82, 105} Alternatively, p80/NS3 can be produced *in trans* by a defective BVDV genome, with a large internal deletion of the structural proteins.¹¹⁹ BVDV isolates have been identified in which p80/NS3 is produced without evidence of gross genomic rearrangements, and mutations have been assumed to be responsible for processing at site 6 (see Figs. 5–7). The diverse collection of CP-BVDV isolates with alternative pathways to produce p80/NS3 is likely to be incomplete, but the unifying theme is always p80/NS3 expression.

In Vitro: Correlates of p80/NS3 Expression. Expression p80/NS3 in BVDV infected cells correlates with induction of cytopathic changes in cells.^{43, 100} These changes could result from a direct interactions of p80/NS3 with cellular macromolecules, with perturbation of cell physiology. Alternatively, p80/NS3 may enhance BVDV replication. The resulting

accumulation and biological activity of viral macromolecules may cause cellular pathology. A third scenario to be considered involves a combination of both previous mechanisms. Available data are consistent with possible involvement of enzymatic activities of p80/NS3, helicase, and protease in cellular pathology, as itemized below:

1. p80/NS3 is consistently present in CP-BVDV-infected cells. Conversely, p80/NS3 is absent in NCP-BVDV.^{43, 100}
2. Sheep and swine pestiviruses display NCP phenotypes and do not produce p80/NS3 in infected cells. Cytopathic border disease isolates have been described; all of these also produce p80/NS3.⁴⁸
3. The 2A protease of poliovirus enhances translation of poliovirus RNA and several other RNAs that have internal ribosome entry sites (IRES). Enhancement of translation by 2A required the active protease; the effect is independent of its ability to shut off the host by cleaving p220.⁶² p80/NS3 may have a similar effect on BVDV translation, which could enhance RNA accumulation indirectly.
4. Mutations in the 2C protein of picornaviruses, shown to be a helicase, can increase virus growth dramatically in MRC-5 cells. A helicase can participate in the modulation of viral growth efficiency. It is possible that the helicase activity of p80/NS3 up regulates growth differentially by lacking the p54/NS2 attached to its N-terminus.^{50, 75, 112}

In Vivo Readout for CP-BVDV: Mucosal Disease. Two key elements are crucial in mucosal disease pathogenesis: first, the immunotolerance to BVDV; second, a CP-BVDV strain, antigenically identical or similar to the NCP-BVDV responsible for tolerance and persistence.^{12, 19, 20, 86} CP-BVDV-induced cytopathology is certainly an *in vitro* phenomenon. The extent to which it takes place *in vivo* is unknown. The excellent correlation between BVDV cytopathology *in vitro* and mucosal disease suggests that cytopathology, alone, or cytopathology in conjunction with additional as yet unknown traits, is required for disease induction. Evolution of viruses that cause mucosal disease in persistently infected animals occurs over a period of several years. Cytopathogenicity may be only one of the traits that are selected in the course of persistent infection. Concomitant selection of other traits is possible. Mucosal disease could be viewed simply as a whole animal readout of a BVDV that evolved to acquire a cytopathic phenotype. Alternatively, cytopathogenicity may be a necessary trait but may not be sufficient for a virus to cause mucosal disease.

ACKNOWLEDGMENTS

The author appreciates the many contributions of his colleagues in the Department of Veterinary and Biomedical Sciences. The author thanks Dr. Vicky Cooper for helpful discussions, as well as Mrs. Leticia Martin and Ms. Beverly Shelbourn for help with bibliographic references.

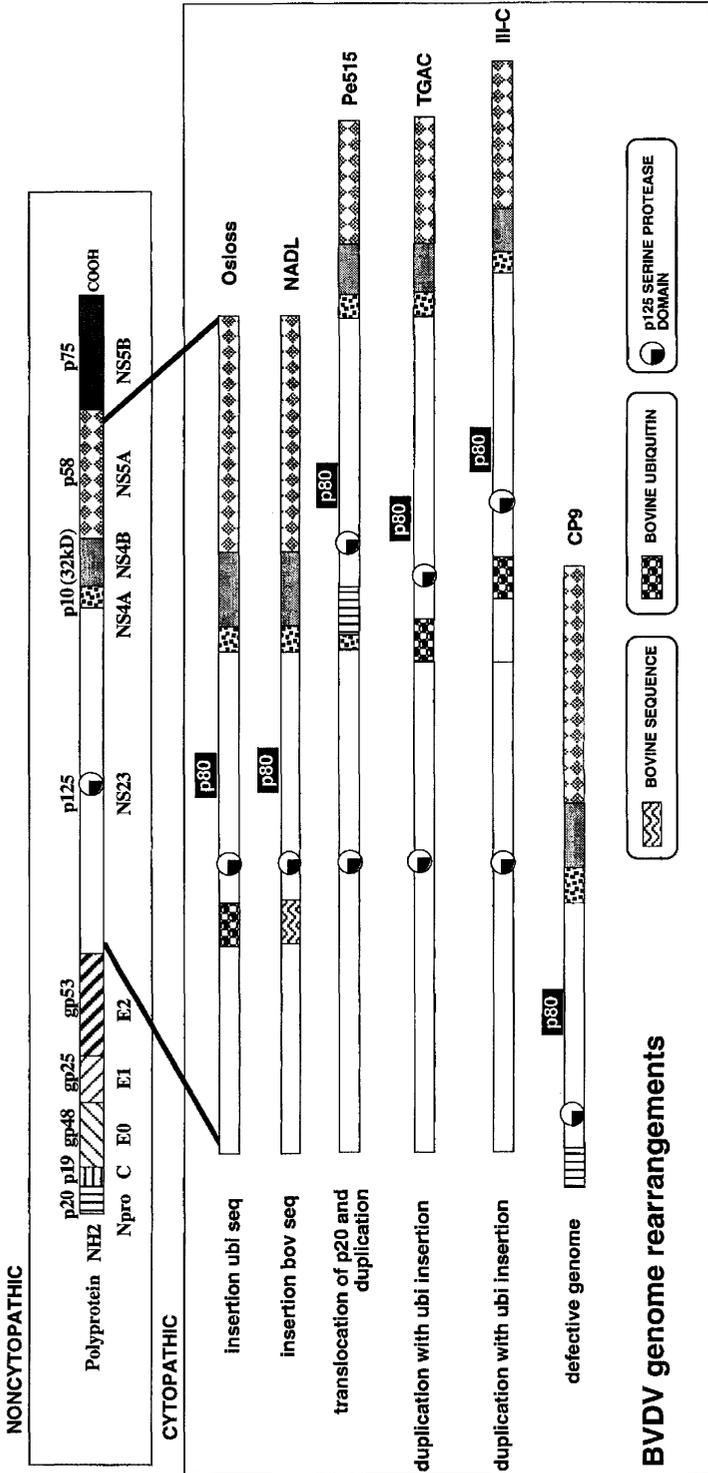


Figure 7. Examples of multiple natural rearrangements in CP-BVDV genomes isolated from cases of MD thought to be responsible for expression of p80/NS3 linked to the ability to induce cytopathology.

References

1. Akkina RK: Analysis of immunogenic determinants of bovine viral diarrhoea virus. St Paul, MN, University of Minnesota, Doctoral Thesis, 1982
2. Akkina RK: Pestivirus bovine viral diarrhoea virus polypeptides: Identification of new precursor proteins and alternative cleavage pathways. *Virus Res* 19:67, 1991
3. Akkina RK, Raisch KP: Intracellular virus-induced polypeptides of pestivirus border disease virus. *Virus Res* 16:95, 1990
4. Bazan JF, Fletterick RJ: Detection of a trypsin-like serine protease domain in flaviviruses and pestiviruses. *Virology* 171:637, 1989
5. Becher P, Shannon AD, Tautz N, et al: Molecular characterization of border disease virus, a pestivirus from sheep. *Virology* 198:542, 1994
6. Bielefeldt-Ohman H, Ronsholt L, Bloch B: Demonstration of bovine viral diarrhoea virus in peripheral blood mononuclear cells of persistently infected, clinically normal cattle. *J Gen Virol* 68:1971, 1987
7. Bissey LL, Williams AK, Bolin S, et al: Comparison of cytopathic and noncytopathic isolates of bovine viral diarrhoea virus by oligonucleotide fingerprinting. *J Vet Diagn Invest* 3:16, 1991
8. Bolin S, Moennig V, Kelso GN, et al: Monoclonal antibodies with neutralizing activity segregate isolates of bovine viral diarrhoea virus into groups. Brief report. *Arch Virol* 99:117, 1988
9. Bolin SR: Viral and viral protein specificity of antibodies induced in cows persistently infected with noncytopathic bovine viral diarrhoea virus after vaccination with cytopathic bovine viral diarrhoea virus. *Am J Vet Res* 49:1040, 1988
10. Bolin SR, Littledike ET, Ridpath JF: Serologic detection and practical consequences of antigenic diversity among bovine viral diarrhoea viruses in a vaccinated herd. *Am J Vet Res* 52:1033, 1991
11. Bolin SR, Matthews PJ, Ridpath JF: Methods for detection and frequency of contamination of fetal calf serum with bovine viral diarrhoea virus and antibodies against bovine viral diarrhoea virus. *J Vet Diagn Invest* 3:199, 1991
12. Bolin SR, McClurkin AW, Cutlip RC, et al: Severe clinical disease induced in cattle persistently infected with noncytopathic bovine viral diarrhoea virus by superinfection with cytopathic bovine viral diarrhoea virus. *Am J Vet Res* 46:573, 1985
13. Bolin SR, Ridpath JF: Specificity of neutralizing and precipitating antibodies induced in healthy calves by monovalent modified-live bovine viral diarrhoea virus vaccines. *Am J Vet Res* 50:817, 1989
14. Bolin SR, Ridpath JF: Differences in virulence between two noncytopathic bovine viral diarrhoea viruses in calves. *Am J Vet Res* 53:2157, 1992
15. Bolin SR, Ridpath JF, Black J, et al: Survey of cell lines in the American Type Culture Collection for bovine viral diarrhoea virus. *J Virol Methods* 48:211, 1994
16. Boulanger D, Dubuisson J, Pastoret P: Studies on the neutralization mechanism of bovine viral diarrhoea virus. Second Symposium on Ruminant Pestivirus, Annecy, France, 1992, p. 157
17. Boulanger D, Waxweiler S, Karelle L, et al: Characterization of monoclonal antibodies to bovine viral diarrhoea virus: Evidence of a neutralizing activity against gp48 in the presence of goat anti-mouse immunoglobulin serum. *J Gen Virol* 72:1195, 1991
18. Brock KV, Deng R, Riblet SM: Nucleotide sequencing of 5' and 3' termini of bovine viral diarrhoea virus by RNA ligation and PCR. *J Virol Methods* 38:39, 1992
19. Brownlie J: Clinical aspects of the bovine virus diarrhoea/mucosal disease complex in cattle. *In Practice* 7:195, 1985
20. Brownlie J, Clarke MC, Howard MC: Experimental production of fatal mucosal disease in cattle. *Vet Rec* 114:535, 1984
21. Bukh J, Purcell RH, Miller RH: Sequence analysis of the 5' noncoding region of hepatitis C virus. *Proc Natl Acad Sci U S A* 89:4942, 1992
22. Chambers TJ, Hahn CS, Galler R, et al: Flavivirus genome organization, expression, and replication. *Ann Rev Microbiol* 44:649, 1990
23. Choo QL, Richman KH, Han JH, et al: Genetic organization and diversity of the hepatitis C virus. *Proc Natl Acad Sci U S A* 88:2451, 1991

24. Clark EG, Norman GR, Janzen ED: Pathologic and virologic findings associated with bovine virus diarrhoea-mucosal disease virus infection in neonatal beef calves in Saskatchewan. *In* Proceedings of the Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, 1985, p. 321
25. Collett MS: Molecular genetics of pestiviruses. *Comp Immunol Microbiol Infect Dis* 15:145, 1992
26. Collett MS, Larson R, Belzer SK, et al: Proteins encoded by bovine viral diarrhoea virus: The genomic organization of a pestivirus. *Virology* 165:200, 1988
27. Collett MS, Moennig V, Horzinek MC: Recent advances in pestivirus research. *J Gen Virol* 70:253, 1989
28. Corapi W, French T, Dubovi E: Severe thrombocytopenia in young calves experimentally infected with noncytopathic bovine viral diarrhoea virus. *J Virol* 63:3934, 1989
29. Corapi WV, Donis RO, Dubovi EJ: Characterization of a panel of monoclonal antibodies and their use in the study of the antigenic diversity of bovine viral diarrhoea virus. *Am J Vet Res* 51:1388, 1990
30. Coria MF, Schmerr MJF, McClurkin AW: Characterization of the major structural proteins of purified bovine viral diarrhoea virus. *Arch Virol* 76:335, 1983
31. Darbyshire JH: A serological relationship between swine fever and mucosal disease of cattle. *Vet Rec* 72:331, 1960
32. De Moerlooze L, Desport M, Renard A, et al: The coding region for the 54-kDa protein of several pestiviruses lacks host insertions but reveals a "zinc finger-like" domain. *Virology* 177:812, 1990
33. De Moerlooze L, Lecomte C, Brown-Shimmer S, et al: Nucleotide sequence of the bovine viral diarrhoea virus Osloss strain: Comparison with related viruses and identification of specific DNA probes in the 5' untranslated region. *J Gen Virol* 74:1433, 1993
34. Deng R, Brock KV: Molecular cloning and nucleotide sequence of a pestivirus genome, noncytopathic bovine viral diarrhoea virus strain SD-1. *Virology* 191:867, 1992
35. Deng R, Brock KV: 5' and 3' untranslated regions of pestivirus genome: Primary and secondary structure analyses. *Nucleic Acids Res* 21:1949, 1993
36. Diderholm H, Dinter Z: Infectious RNA derived from Bovine Virus Diarrhoea Virus. *Zentbl Bakteriol Abt I* 201:270, 1966
37. Dinter Z: Relationship between bovine virus diarrhoea virus and hog cholera virus. *Zentbl Bakteriol Abt I* 188:475, 1963
38. Domingo E: Genetic variation and quasi-species. *Curr Opin Genet Dev* 2:61, 1992
39. Domingo E, Martinez SE, Sobrino F, et al: The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: Biological relevance—A review. *Gene* 40:1, 1985
40. Donis R, Corapi W, Dubovi E: Bovine viral diarrhoea and their antigenic analyses. *Arch Virol* 3(suppl):29, 1991
41. Donis RO, Corapi W, Dubovi EJ: Neutralizing monoclonal antibodies to bovine viral diarrhoea virus bind to the 56K to 58K glycoprotein. *J Gen Virol* 69:77, 1988
42. Donis RO, Dubovi EJ: Characterization of bovine viral diarrhoea-mucosal disease virus-specific proteins in bovine cells. *J Gen Virol* 68:1597, 1987
43. Donis RO, Dubovi EJ: Differences in virus-induced polypeptides in cells infected by cytopathic and noncytopathic biotypes of bovine virus diarrhoea-mucosal disease virus. *Virology* 158:168, 1987
44. Donis RO, Dubovi EJ: Glycoproteins of bovine viral diarrhoea-mucosal disease virus in infected bovine cells. *J Gen Virol* 68:1607, 1987
45. Donis RO, Dubovi EJ: Molecular specificity of the antibody responses of cattle naturally and experimentally infected with cytopathic and noncytopathic bovine viral diarrhoea virus biotypes. *Am J Vet Res* 48:1549, 1987
46. Drummond DR, Armstrong J, Colman A: The effect of capping and polyadenylation on the stability, movement and translation of synthetic messenger RNAs in *Xenopus* oocytes. *Nucleic Acids Res* 13:7375, 1985
47. Dubovi EJ: Genetic diversity and BVD virus. *Comp Immunol Microbiol Infect Dis* 15:155, 1992
48. Dutia BM, Entrican G, Nettleton PF: Cytopathic and non-cytopathic biotypes of

- border disease virus induce polypeptides of different molecular weight with common antigenic determinants. *J Gen Virol* 71:1227, 1990
49. Eigen M: Self-organization of matter and the evolution of biological macromolecules. *Naturwissenschaften* 58:465, 1971
 50. Emerson SU, Huang YK, McRill C, et al: Mutations in both the 2B and 2C genes of hepatitis A virus are involved in adaptation to growth in cell culture. *J Virol* 66:650, 1992
 51. Fernelius AL, Lambert G, Hemmes GJ: Bovine viral diarrhea virus-host cell interactions: Adaptation and growth of virus in cell lines. *Am J Vet Res* 30:1561, 1969
 52. Fernelius AL, Lambert G, Packer RA: Bovine viral diarrhea virus-host cell interactions: Adaptation, propagation, modification, and detection of virus in rabbits. *Am J Vet Res* 30:1541, 1969
 53. Fernelius AL, Ritchie AE: Mixed infections or contaminations of bovine viral diarrhea virus with infectious bovine rhinotracheitis virus. *Am J Vet Res* 27:241, 1966
 54. Flores E, Donis R: Isolation of a mutant MDBK cell line resistant to bovine viral diarrhea virus infection due to a block in viral entry. *Virology* 208:565, 1995
 55. Francki R, Fauquet C, Knudson D, et al: Classification and nomenclature of viruses. Fifth Report of the ICTV. New York, Springer-Verlag, 1991
 56. Gillespie JH, Baker JA, McEntee K: A cytopathogenic strain of virus diarrhea virus. *Cornell Vet* 50:73, 1960
 57. Gorbalenya AE, Donchenko AP, Koonin EV, et al: N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. *Nucleic Acids Res* 17:3889, 1989
 58. Gorbalenya AE, Koonin EV, Donchenko AP, et al: Two related superfamilies of putative helicases involved in replication, recombination, repair, and expression of DNA and RNA genomes. *Nucleic Acids Res* 17:4713, 1989
 59. Gorbalenya AE, Koonin EV, Lai MM: Putative papain-related thiol proteases of positive-strand RNA viruses. Identification of rubi- and aphthovirus proteases and delineation of a novel conserved domain associated with proteases of rubi-, alpha- and coronaviruses. *FEBS Lett* 288:201, 1991
 60. Greiser-Wilke I, Haas L, Dittmar K, et al: RNA insertions and gene duplications in the nonstructural protein p125 region of pestivirus strains and isolates in vitro and in vivo. *Virology* 193:977, 1993
 61. Greiser-Wilke I, Liebler E, Haas L, et al: Distribution of cytopathogenic and noncytopathogenic bovine virus diarrhea virus in tissues from a calf with experimentally induced mucosal disease using antigenic and genetic markers. *Arch Virol* 7(suppl):295, 1993
 62. Hambidge SJ, Sarnow P: Translational enhancement of the poliovirus 5' noncoding region mediated by virus-encoded polypeptide 2A. *Proc Natl Acad Sci U S A* 89:10272, 1992
 63. Heinz FX: Comparative molecular biology of flaviviruses and hepatitis C virus. *Arch Virol* 4(suppl):163, 1992
 64. Hermodsson S, Dinter Z: Properties of bovine virus diarrhea virus. *Nature* 194:893, 1962
 65. Horzinek MC: Nonarbo togavirus infections of animals: Comparative aspects and diagnosis. In *Comparative Diagnosis of Viral Diseases*. New York, Academic Press, 1981, p. 442
 66. Horzinek MC, Mussgay M: Studies on the substructure of togaviruses. Effect of urea, deoxycholate and saponin on the Sindbis virion. *Arch Ges Virusforsch* 33:296, 1971
 67. Hulst MM, Himes G, Newbigin E, et al: Glycoprotein E2 of classical swine fever virus: expression in insect cells and identification as a ribonuclease. *Virology* 200:558, 1994
 68. Iizuka N, Najita L, Franzusoff A, et al: Cap-dependent and cap-independent translation by internal initiation of mRNAs in cell extracts prepared from *Saccharomyces cerevisiae*. *Mol Cell Biol* 14:7322, 1994
 69. Khudiyakov YE, Fields HA, Favorov MO, et al: Synthetic gene for the hepatitis C virus nucleocapsid protein. *Nucleic Acids Res* 21:2747, 1993
 70. Koonin EV, Dolja VV: Evolution and taxonomy of positive-strand RNA viruses:

- Implications of comparative analysis of amino acid sequences. *Crit Rev Biochem Mol Biol* 28:375, 1993
71. Laude H: Improved method for the purification of hog cholera virus grown in tissue culture. *Arch Virol* 54:41, 1977
 72. Laude H: Comparative hydrodynamic properties of the pestivirus genus. *Arch Virol* 62:347, 1979
 73. Lee HJ, Shieh CK, Gorbalenya AE, et al: The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. *Virology* 180:567, 1991
 74. Lee KM, Gillespie JH: Propagation of virus diarrhea virus of cattle in tissue culture. *Am J Vet Res* 18:952, 1957
 75. Li JP, Baltimore D: Isolation of poliovirus 2C mutants defective in viral RNA synthesis. *J Virol* 62:4016, 1988
 76. Liess B: Pathogenesis and epidemiology of hog cholera. *Ann Rech Vet* 18:139, 1987
 77. Luther PD, Stott EJ, Jebbett J: Virus contamination of bovine testis cell cultures. *Res Vet Sci* 30:251, 1981
 78. McClurkin AW, Bolin SR, Coria MF: Isolation of cytopathic and noncytopathic bovine viral diarrhea virus from the spleen of cattle acutely and chronically affected with bovine viral diarrhea. *J Am Vet Med Assoc* 186:568, 1985
 79. Meyers G, Rumenapf T, Thiel HJ: Molecular cloning and nucleotide sequence of the genome of hog cholera virus. *Virology* 171:555, 1989
 80. Meyers G, Rumenapf T, Thiel HJ: Ubiquitin in a togavirus [letter]. *Nature* 341:491, 1989
 81. Meyers G, Tautz N, Dubovi EJ, et al: Viral cytopathogenicity correlated with integration of ubiquitin-coding sequences. *Virology* 180:602, 1991
 82. Meyers G, Tautz N, Stark R, et al: Rearrangement of viral sequences in cytopathogenic pestiviruses. *Virology* 191:368, 1992
 83. Miller RH, Purcell RH: Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. *Proc Natl Acad Sci U S A* 87:2057, 1990
 84. Moennig V: Dissertation. Hannover, Germany, Veterinary School, 1971
 85. Moennig V, Bolin SR, Coulibaly CO, et al: Studies of the antigen structure of pestiviruses using monoclonal antibodies. *Dtsch Tierarztl Wochenschr* 94:572, 1987
 86. Moennig V, Frey HR, Liebler E, et al: Reproduction of mucosal disease with cytopathogenic bovine viral diarrhea virus selected in vitro. *Vet Rec* 127:200, 1990
 87. Moennig V, Plagemann PG: The pestiviruses. *Adv Virus Res* 41:53, 1992
 88. Molla A, Jang SK, Paul AV, et al: Cardioviral internal ribosomal entry site is functional in a genetically engineered dicistronic poliovirus. *Nature* 356:255, 1992
 89. Moorman RJM, Warmerdam PAM, Van Der Meer B, et al: Molecular cloning and nucleotide sequence of hog cholera virus strain Brescia and the mapping of the genomic region encoding envelope protein E1. *Virology* 177:184, 1990
 90. Nuttall PA, Luther PD, Stott EJ: Viral contamination of bovine foetal serum and cell cultures. *Nature (London)* 266:835, 1977
 91. Olafson P, MacCallum AD, Fox A: An apparently new transmissible disease of cattle. *Cornell Vet* 36:205, 1946
 92. Olafson P, Rickard CG: Further observations on the virus diarrhea (new transmissible disease) of cattle. *Cornell Vet* 37:104, 1947
 93. Onyekaba C, Fahrman J, Bueon L, et al: Comparison of five cell lines for the propagation of bovine viral diarrhea and infectious bovine rhinotracheitis viruses. *Microbiologica* 10:311, 1987
 94. Paton DJ, Ibata G, Edwards S, et al: An ELISA detecting antibody to conserved pestivirus epitopes. *J Virol Methods* 31:315, 1991
 95. Paton DJ, Lowings JP, Barrett AD: Epitope mapping of the gp53 envelope protein of bovine viral diarrhea virus (published erratum appears in *Virology* 191:1013, 1992). *Virology* 190:763, 1992
 96. Pellerin C, van den Hurk J, Lecomte J, et al: Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. *Virology* 203:260, 1994

97. Pestova TV, Hellen CU, Wimmer E: A conserved AUG triplet in the 5' nontranslated region of poliovirus can function as an initiation codon in vitro and in vivo. *Virology* 204:729, 1994
98. Plant JW, Littlejohns IR, Gardiner AC, et al: Immunological relationship between border disease, mucosal disease and swine fever. *Vet Record* 92:455, 1973
99. Plateau E, Vannier P, Tillon J: A typical hog cholera infection: Viral isolation and clinical study of in utero transmission. *Am J Vet Res* 41:2012, 1980
100. Pocock DH, Howard CJ, Clarke MC, et al: Variation in the intracellular polypeptide profiles from different isolates of bovine virus diarrhoea virus. *Arch Virol* 94:43, 1987
101. Poole T, Wang C, Popp R, et al: Pestivirus translation initiation occurs by internal ribosome entry. *Virology* 206:750, 1995
102. Porterfield JS, Casals J, Chumakov MP, et al: *Togaviridae*. *Intervirology* 9:129, 1978
103. Potts BJ, Sawyer M, Shekarchi IC, et al: Peroxidase-labeled primary antibody method for detection of pestivirus contamination in cell cultures. *J Virol Method* 26:119, 1989
104. Purchio AF, Larson R, Torborg LL, et al: Cell-free translation of bovine viral diarrhoea virus RNA. *J Virol* 52:973, 1984
105. Qi F, Ridpath JF, Lewis T, et al: Analysis of the bovine viral diarrhoea virus genome for possible cellular insertions. *Virology* 189:285, 1992
106. Rebhun W, French TW, Perdrizet JA, et al: Thrombocytopenia associated with acute bovine virus diarrhoea infection in cattle. *J Vet Intern Med* 3:42, 1989
107. Renard A, Dina D, Martial J: Vaccines and diagnostics derived from bovine viral diarrhoea virus. European Patent Application No.86870095. Pub. No. 0208672, 1/14-87, 1987
108. Ridpath J, Bolin S, Dubovi E: Segregation of Bovine Viral Diarrhoea Virus into genotypes. *Virology* 205:66, 1994
109. Ridpath JF, Lewis TL, Bolin SR, et al: Antigenic and genomic comparison between non-cytopathic and cytopathic bovine viral diarrhoea viruses isolated from cattle that had spontaneous mucosal disease. *J Gen Virol* 72:725, 1991
110. Risatti G, Smith C, Donis RO. Mutations leading to thermosensitive biotype switch in bovine viral diarrhoea virus. 13th annual meeting of the American Society of Virology, University of Wisconsin, Madison, Wisconsin, July 1994
111. Ritchie AE, Fernelius AL: Characterization of bovine viral diarrhoea viruses. V. Morphology of characteristic particles studied by electron microscopy. *Arch Virol* 28:369, 1969
112. Rodriguez PL, Carrasco L: Poliovirus protein 2C has ATPase and GTPase activities. *J Biol Chem* 268:8105, 1993
113. Rossi CR, Bridgman CR, Kiesel GK: Viral contamination of bovine fetal lung cultures and bovine fetal serum. *Am J Vet Res* 41:1680, 1980
114. Schneider R, Unger G, Stark R, et al: Identification of a structural glycoprotein of an RNA virus as a ribonuclease. *Science* 261:1169, 1993
115. Sullivan DG, Chang GJ, Trent DW, et al: Nucleotide sequence analysis of the structural gene coding region of the pestivirus border disease virus. *Virus Res* 33:219, 1994
116. Susa M, Konig M, Saalmuller A, et al: Pathogenesis of classical swine fever: B-lymphocyte deficiency caused by hog cholera virus. *J Virol* 66:1171, 1992
117. Tamura JK, Warrener P, Collett MS: RNA-stimulated NTPase activity associated with the p80 protein of the pestivirus bovine viral diarrhoea virus. *Virology* 193:1, 1993
118. Tautz N, Meyers G, Thiel HJ: Processing of poly-ubiquitin in the polyprotein of an RNA virus. *Virology* 197:74, 1993
119. Tautz N, Thiel HJ, Dubovi EJ, et al: Pathogenesis of mucosal disease: A cytopathogenic pestivirus generated by an internal deletion. *J Virol* 68:3289, 1994
120. Thomas AA, Scheper GC, Kleijn M, et al: Dependence of the adenovirus tripartite leader on the p220 subunit of eukaryotic initiation factor 4F during in vitro translation. Effect of p220 cleavage by foot-and-mouth-disease-virus L-protease on in vitro translation. *Eur J Biochem* 207:471, 1992
121. Torgerson PR, Dalgleish R, Gibbs HA: Mucosal disease in a cow and her suckled calf. *Vet Rec* 125:530, 1989
122. Tsukiyama KK, Iizuka N, Kohara M, et al: Internal ribosome entry site within hepatitis C virus RNA. *J Virol* 66:1476, 1992

123. Vassilev V, Collett M, Donis R. Full-length cDNA clone of Bovine Viral Diarrhea virus producing infectious RNA transcripts. *In* Proceedings of the IV International Congress on Positive Strand RNA Viruses, Utrecht, The Netherlands, 1995
124. Ward ACS, Kaeberle ML: Use of an immunoperoxidase stain for the demonstration of bovine viral diarrhea virus by light and electron microscopies. *Am J Vet Res* 45:165, 1984
125. Warrener P, Collett M: Pestivirus NS3 (p80) protein possesses RNA helicase activity. *J Virol* 69:1720, 1995
126. Wellemans G, Opdenbosch EV: Demonstration of BVD virus (bovine diarrhea virus) in many cell lines. *Ann Rech Vet* 18:99, 1987
127. Wensvoort G, Terpstra C: Bovine viral diarrhoea virus infections in piglets born to sows vaccinated against swine fever with contaminated vaccine. *Res Vet Sci* 45:143, 1988
128. Wensvoort G, Terpstra C, de Kluyver E: Characterization of porcine and some ruminant pestiviruses by cross-neutralization. *Vet Microbiol* 20:291, 1989
129. Wilhelmsen CL, Bolin SR, Ridpath JF, et al: Lesions and localization of viral antigen in tissues of cattle with experimentally induced or naturally acquired mucosal disease, or with naturally acquired chronic bovine viral diarrhea. *Am J Vet Res* 52:269, 1991
130. Wiskerchen M, Collett MS: Pestivirus gene expression: Protein p80 of bovine viral diarrhea virus is a proteinase involved in polyprotein processing. *Virology* 184:341, 1991
131. Xue W, Blecha F, Minocha HC: Antigenic variations in bovine viral diarrhea viruses detected by monoclonal antibodies. *J Clin Microbiol* 28:1688, 1990
132. Xue W, Minocha HC: Identification of the cell surface receptor for bovine viral diarrhoea virus by using anti-idiotypic antibodies. *J Gen Virol* 74:73, 1993
133. Zingg HH, Lefebvre DL, Almazan G: Regulation of poly(A) tail size of vasopressin mRNA. *J Biol Chem* 263:11041, 1988

Address reprint requests to

Ruben O. Donis, MV, PhD
202 VBS

Department of Veterinary and Biomedical Sciences
University of Nebraska-Lincoln
Lincoln, NE 68583-0905