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Understanding the Pathogenesis of Cytopathic and Noncytopathic Bovine Viral Diarrhea Virus Infection Using Proteomics

Mais Ammari[1], Fiona McCarthy[1,2], Bindu Nanduri[1,2],
George Pinchuk[3] and Lesya Pinchuk[1]
*[1]Department of Basic Sciences,
Mississippi State University, Mississippi State, MS
[2]Institute for Genomics, Biotechnology and Biocomputing,
Mississippi State University, Mississippi State, MS
[3]Department of Sciences and Mathematics,
Mississippi University for Women, Columbus, MS
USA*

1. Introduction

Bovine Viral Diarrhea Virus (BVDV) is a single-stranded RNA virus in the *Pestivirus* genus within the *Flaviviridae* family. BVDV infections are seen in all ages and breeds of cattle worldwide and have significant economic impact due to productive and reproductive losses (Houe 2003). Two antigenically distinct genotypes of BVDV exist, type 1 and 2 (Ridpath *et al.* 1994). BVDV of both genotypes may occur as cytopathic (cp) or noncytopathic (ncp) biotypes, classified according to whether or not they produce visible changes in cell culture. Data indicate that cp biotypes of BVDV can actually be created through internal deletion of RNA of ncp biotypes, or through RNA recombination between ncp biotypes (Howard *et al.* 1992). Of the two BVDV biotypes, infection of a fetus by ncp BVDV can result in persistently infected (PI) calf that sheds the virus throughout its life without developing clinical signs of infection. PI animals are the major disseminators of BVDV in the cattle population and have been the cause of severe acute outbreaks (Carman *et al.* 1998). However cp BVDV is associated predominantly with animals that develop mucosal disease (MD), which can be acute, resulting in death within a few days of onset, or chronic, persisting for weeks or months before the afflicted animal dies (Houe 1999).

The interaction of BVDV with its host has several unique features, most notably the capacity to infect its host either transiently or persistently (Liebler-Tenorio *et al.* 2002; Bendfeldt S 2007). Initially the virus binds to CD46, a complement receptor expressed on lymphoid cells, monocytes, macrophages and dendritic cells and serving as a "magnet" for several viral and bacterial pathogens (Cattaneo 2004). Upon entry, the virus replicates and spreads in the lymphatic system, impairing the immunity of the infected animal, particularly antigen presenting cells (APC) function and production of interferons (IFN). Cytopathic BVDV biotype but not ncp biotype (Schweizer & Peterhans 2001) is implicated in the induction of apoptosis (Zhang *et al.* 1996; Schweizer & Peterhans 1999; Grummer *et al.* 2002; Jordan *et al.*

2002), and the existence of the two antigenically related biotype 'pairs' makes BVDV an important model for virus-induced apoptosis. In addition, cp BVDV readily trigger IFN type-I whereas infection with ncp BVDV fails to induce IFN generation (Peterhans *et al.* 2003). Also, BVDV has been reported to modulate functions of immune cells after infection *in vitro* including increased production of nitric oxide from infected macrophages (Adler *et al.* 1994), decreased production of TNF-α from activated macrophages (Adler *et al.* 1994), inhibited phagocytosis of alveolar macrophages (Brewoo *et al.* 2007) and decreased T-stimulatory ability of monocytes (Glew *et al.* 2003). Phagocytosis and macropinocytosis antigen uptake mechanisms play a crucial role in the innate immune responses by clearing pathogens at sites of infection. The endothytic pathways are also important early steps in triggering the adaptive immune responses which require processing of bacterial and viral pathogens and presentation of their antigens to CD4+ and CD8+ T cells (Boyd *et al.* 2004).

Since BVDV viruses are able to affect virtually all organs and systems in the body, including the innate and the adaptive immune system, it is important to know the mechanistic framework for the viral-host interactions in the complex etiology of the disease. The availability of BVDV genome sequence makes it a suitable target for genome-wide analyses. However, the corresponding viral proteome, the alterations in host proteomes upon BVDV infection and the dynamic nature of the BVDV proteins remain largely unknown.

New developments in comparative and quantification proteomics technology, especially mass spectrometry (MS), enabled a more comprehensive characterization of virions, protein location, protein isoforms and post-translational modifications, as well as protein-protein interactions involved in virus-host dynamics. One example on the application of these new advances is in the detection of virion protein composition that helped in identifying the role of specific viral proteins during infection. Since BVDV is an enveloped virus, it has a considerable potential to incorporate both viral and host proteins into its membrane as well as into the envelope, and these can be present at low levels, making their detection difficult using traditional methods. In recent viral proteomics studies, several different MS approaches (e.g., matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF) mass spectrometry and LC-MS/MS) were successfully used to analyze the composition of a variety of virions, leading to the identification of previously unknown components of viral particles. For example, two enveloped viruses, SARS and HIV-1 were analyzed by these techniques and the investigators were able to confirm and identify virion proteins (Maxwell & Frappier 2007). Accurate identification of BVDV virion proteins is possible using these methods.

In this review, we discuss the work that has been done to date using proteomic-related approaches to understand BVDV viral protein structure, viral protein-protein interactions and viral-host protein interactions. At the end of each section we refer to some examples of new proteomics approaches that have been successfully used for different viral studies that can be applied for studying BVDV to develop a better understanding of its pathogenesis. We also include the effects of BVDV viral infection on host cell proteome where we place particular emphasis on MS-based approaches, highlighting how these new approaches facilitated the understanding of BVDV pathogenesis at a genomic scale.

2. Structural proteomics

The availability of genome sequences coupled with advances in molecular and structural biology guided the development of structural proteomics. Availability of three-dimensional

structure information elucidates protein function and identifies targets for the attenuation of virus's replication. Currently, the Protein Data Bank (PDB) (H.M. Berman 2000) contains eight structures for BVDV-encoded proteins, all of which were solved in the past seven years (Table 1). In contrast, the whole *Flaviviridae* family has 312 protein structures.

Protein	PDB accession number	Type of structural data	Release date	Reference
RdRp (BVDV CP7-R12 RdRp (residues 3189-3907))	2CJQ	X-RAY DIFFRACTION	2006-07-19	(Choi *et al.* 2006)
*NS5A (NMR structure of the in-plane membrane anchor domain [1-28] of the monotopic NS5A of BVDV)	2AJJ	SOLUTION NMR	2005-08-23	(Sapay *et al.* 2006)
*NS5A (NMR structure of the in-plane membrane anchor domain [1-28] of the monotopic NS5A from the BVDV)	2AJM	SOLUTION NMR	2005-08-23	(Sapay *et al.* 2006)
*NS5A (NMR structure of the in-plane membrane anchor domain [1-28] of the monotopic NS5A from the BVDV)	2AJN	SOLUTION NMR	2005-08-23	(Sapay *et al.* 2006)
*NS5A (NMR structure of the in-plane membrane anchor domain [1-28] of the monotopic NS5A from the BVDV)	2AJO	SOLUTION NMR	2005-08-23	(Sapay *et al.* 2006)
RdRp (Crystal structure of RdRp construct 1 (residues 71-679) from BVDV)	1S48	X-RAY DIFFRACTION	2004-04-06	(Choi *et al.* 2004)
RdRp (Crystal Structure of RdRp construct 1 (residues 71-679) from BVDV complexed with GTP)	1S49	X-RAY DIFFRACTION	2004-04-06	(Choi *et al.* 2004)
RdRp (Crystal Structure of RdRp construct 2 (Residues 79-679) from BVDV)	1S4F	X-RAY DIFFRACTION	2004-04-06	(Choi *et al.* 2004)

Table 1. BVDV proteins structure. *For details on the differences between the four structures see indicated reference

BVDV genome is a positive-sense ssRNA of approximately 12.6-kb in length (Meyers et al. 1997; Brett D. Lindenbach 2007). The BVDV genome comprises a 5′ and 3′ untranslated regions (UTR), which flank a single open reading frame (ORF) (Collett et al. 1988; Brett D. Lindenbach 2007). The BVDV genome is translated into a single polyprotein NH2-N^pro-C-

E^rns-E1-E2-P7-NS2-3-NS4A-NS4B-NS5A-NS5B-COOH (Collett *et al.* 1988; Meyers *et al.* 1997; Brett D. Lindenbach 2007) (Figure 1). Upon synthesis, by a combination of host and viral proteases, the BVDV polyprotein is processed into at least four structural (C, E^rns, E1, E2) and six non-structural (NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins required for viral assembly and replication.
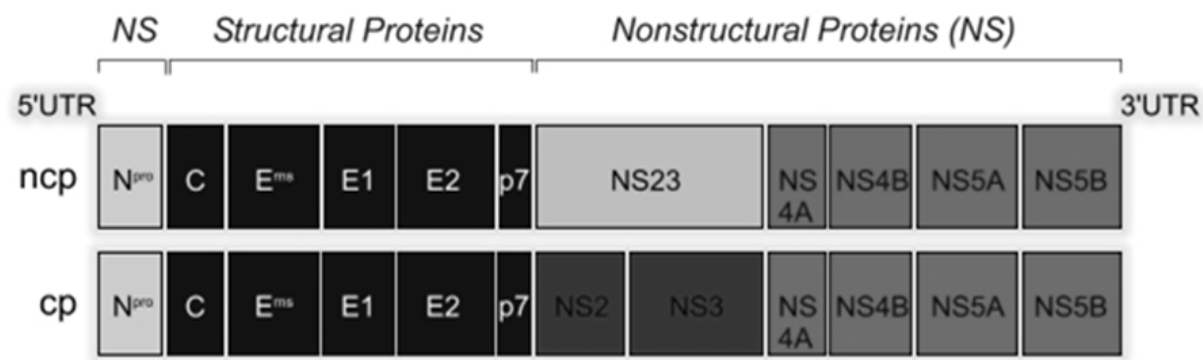

Fig. 1. BVDV genome structure

Among the NS proteins, NS5B has been shown to be the BVDV RNA-dependent RNA polymerase (RdRp) which is responsible for genomic replication as a part of larger, membrane associated, replicase complex (Brett D. Lindenbach 2007). Since BVDV uses a primer-independent (*de novo*) mechanism for RNA replication, its RNA polymerase requires GTP for initiating RNA synthesis (Brett D. Lindenbach 2007). 2.9 °A X-ray crystallography structural data of BVDV strain NADL RdRp revealed that it possesses a GTP N-terminal domain and identified the GTP-specific binding site required for *de novo* initiation (Choi *et al.* 2004). Comparison of the 2.6 °A X-ray crystal structure of BVDV CP7-R12, a BVDV CP7 polymerase recombinant with a single amino acid duplication of Asn438 (Choi *et al.* 2006), with the NADL BVDV polymerase showed that the alterations in the RdRp of the CP7-R12 derived mutant viruses could be allocated to a large fragment of the N-terminal domain indicating the role of this domain in the translocation of the template during catalysis. In addition, the study showed the formation of an unstable loop due to the insertion of an additional Asn438 in CP7-R12, which may account for the low replication activity of the mutant polymerase *in vivo* (Gallei *et al.* 2004).

BVDV NS5A function is not well determined. Three-dimensional NMR structure analyses of the membrane anchor (1-28) of NS5A from BVDV performed either in 50% TFE or in SDS micelles to mimic the membrane environment revealed that the N-terminal membrane anchor of NS5A includes a long amphipathic α-helix (aa 5-25). It interacts in-plane with the membrane interface and is divided into two portions separated by a flexible region centred around residue Gly19 (Sapay *et al.* 2006). The amphipathic α-helix exhibited a hydrophobic side buried in the membrane and a polar side accessible from the cytosol. These data were also confirmed and supported by molecular dynamic simulation at a water-dodecane interface. Despite the lack of amino acid sequence similarity, this amphipathic α-helix shows a common structural feature with that of the Hepatitis C virus (HCV). The phosphorylation state of NS5A is believed to be vital for HCV replication complex.

In addition, on the basis of sequence alignments of HCV and BVDV NS5A proteins, four cysteine residues involved in zinc binding were identified. BVDV secondary structure assignments of these were determined by computer prediction with the PSIPRED algorithm (Tellinghuisen *et al.* 2006).

Due mostly to solubility issues, generating structures using NMR and X-ray crystallography have been successful for only a small fraction of viral proteins. In contrast, the recent use of high-throughput approaches for multiple viral proteins structures and functions, for example, SARS virus enabled the investigators to elucidate the viral protein structure with high resolution.

3. Protein interactions

Physical interactions of viral proteins between each other and with their host proteins are important in allowing the pathogen to enter the host cell, manipulate host cellular processes, replicate and infect other cells. Identifying protein-protein interactions during the course of infection enables researchers to better understand the role of host-pathogen interactions and how these interactions affect infection, disease progression and the host immune response. Since interaction of intracellular infectious agents like BVDV with their host cells are mainly at the protein level, proteomics is the most suitable tool for investigating these interactions.

3.1 Virus protein-protein interactions

Physical association of viral proteins is important in the initial virus-host interaction and immune response against structural proteins. For example, by co- and sequential immuno precipitation a direct interaction of E^{rns} with E2 was reported very early after translation and showed to form a covalently linked heterodimer, which is later stabilized by disulfide bonds (Lazar *et al.* 2003). This interaction exists in both cp BVDV-infected MDBK cells and secreted virions.

Various cellular and viral processes are dependent on phosphorylation and dephosphorylation of specific proteins. Phosphorylation of multiple Flavivirus NS5 proteins correlates with subcellular localization and ability to associate with NS3. Studies of virion morphogenesis by immunoprecipitation show that the BVDV NS5A is phosphorylated by its associated serine/threonine kinase (Reed *et al.* 1998). Phosphorylation of NS5A may play a role in BVDV life cycle.

Analysis by radioimmunoprecipitation assays followed by SDS-PAGE under nonreducing conditions revealed that the envelope BVDV glycoproteins E1 and E2 interact through a disulfide bond to form a dimer (Weiland *et al.* 1990), which is thought to be a functional complex present on the surfaces of mature virion. In a follow up study, 30 min post-pulse, both E1 and E2 interacted independently and simultaneously with calnexin, an ER chaperone (Branza-Nichita *et al.* 2001). The inhibition of calnexin binding to the envelope proteins by α-glucosidase inhibitors resulted in the misflolding of those proteins and a decrease in the formation of E1-E2 heterodimers (Branza-Nichita *et al.* 2001).

Cp BVDV-infected cells were metabolically labeled and their proteins crosslinked with the cell-permeable and thiol-cleavable cross-linker DSP followed by immunoprecipitation with BVDV protein specific antiserum. Although not sufficient to establish direct protein-protein interactions, results indicate associations between NS3, NS4B, and NS5A (Qu *et al.* 2001).

3.2 Virus-host interacting partners

Identifying interactions between viral proteins and host proteins is important for understanding the mechanisms used by BVDV for successful replication and invasion of their host. Studies used the yeast-two hybrid (Y2H) screening system to screen individual BVDV proteins for host binding partners (Table 2). For example, using Y2H it was

Virus-host protein partner	Method	Reference
NS3-SphK1	Y2H	(Yamane et al. 2009a)
NS5A-α subunit of eEF1A	Y2H	(Johnson et al. 2001)
NS5A-NIBP	Y2H	(Zahoor et al. 2010)
NS2-Jiv	Co-precipitation	(Rinck et al. 2001)

Table 2. BVDV-host interacting proteins

demonstrated that NS3 from BVDV binds to and inhibits the catalytic activity of sphingosine kinase 1 (SphK1). NS3- SphK1 binding enhance BVDV replication and BVDV-induced apoptosis (Yamane *et al.* 2009a).

Using Y2H screening, the α subunit of bovine translation elongation factor 1A (eEF1A) was shown to interact with the NS5A polypeptide of BVDV (Johnson *et al.* 2001). This interaction was further analysed in a cell-free translation system and was found to be conserved among BVDV isolates of both genotypes and biotypes. Cell-free binding studies were done using a chimeric NS5A fused to glutathione S-transferase (GST–NS5A) expressed in bacteria. GST–NS5A bound specifically to both *in vitro* translated and mammalian cell expressed eEF1A. This interaction was suggested to play a role in the replication of BVDV (Johnson *et al.* 2001).

In addition, Y2H screening identified bovine NIK- and IKKβ-binding protein (NIBP), which is involved in protein trafficking and nuclear factor kappa B (NF-κB) signalling in cells (Zahoor *et al.* 2010). The interaction of NS5A with NIBP was confirmed both *in vitro* and *in vivo*. Supporting this data, confocal immunofluorescence results indicate that NS5A co-localized with NIBP on the endoplasmic reticulum in the cytoplasm of BVDV-infected cells. Moreover, the minimal residues of NIBP that interact with NS5A were mapped as aa 597–623. In addition, overexpression of NS5A inhibited NF-κB activation in HEK293 and LB9.K cells. The same study also showed that inhibition of endogenous NIBP by RNAi enhanced virus replication, indicating the importance of NIBP in BVDV pathogenesis. This is the first reported interaction between NIBP and a viral protein, suggesting a novel mechanism whereby viruses may subvert host-cell machinery for mediating trafficking and NF-κB signaling (Zahoor *et al.* 2010).

Efficient generation of NS2-3 cleavage product NS3 is required for the cytopathogenicity of the pestiviruses. Co-precipitation was used to identify the formation of a stable complex between Jiv, a member of the DnaJ-chaperone family, and BVDV nonstructural protein NS2 (Rinck *et al.* 2001). Jiv has the potential to induce in *trans* cleavage of NS2-3.

While Y2H data is commonly used for identifying protein-protein interaction, results of this technique are dependent on the library screened, the relative representation of each cDNA and the expression level of individual proteins. Also, immunoprecipitation approaches are dependent on the availability of high specificity and affinity antibodies. An alternative approach for identifying protein interaction is the use of tandem and column affinity followed by MS or sequencing analysis. These methods require a specific concentration and a highly purified target protein.

Also, the recent effort for the integration of experimentally established host-pathogen protein-protein interactions for several pathogens in public databases allows for cross-

pathogen comparisons and the prediction of these interactions. Databases, for examples, VirHostNet, MINT and HPIDB, allow an investigator to search for homologous host-pathogen interactions and also to get a list of all host-pathogen protein-protein interactions available.

4. Virus-induced changes in the cellular proteome

Studying how viral infection or expression of specific viral proteins affects the expression of the host cell proteome provides insight into metabolic processes and critical regulatory events of the host cell. Although there have been multiple, comprehensive studies to profile viral induced changes in bovine cells at the transcriptional level in response to BVDV infection using microarrays (Werling *et al.* 2005; Maeda *et al.* 2009; Yamane *et al.* 2009b), these observed changes do not always correspond to changes at the protein level. Many viral proteins affect protein turnover without affecting the transcription rate of the protein. Thus, there will always be a need to determine changes at the protein level.

While both bovine and BVDV genome sequences are available, only a few studies have attempted a comprehensive survey of BVDV-induced host protein changes. These studies used MS-based approaches to identify BVDV-induced cellular proteome changes, focusing on host immune changes, which are assessed by comparing protein profiles before and after BVDV infection (Pinchuk *et al.* 2008; Lee *et al.* 2009; Ammari *et al.* 2010) (Figure 2). Proteome coverage (i.e. the proportion of the predicted proteome identified as expressed) is increased by the combined use of differential detergent fractionation (DDF) (McCarthy *et al.* 2005) of infected cells followed by multidimensional protein identification technology (MudPIT) for protein identification. DDF yields four electrophoretically distinct fractions (McCarthy *et al.* 2005). MudPIT was done using strong cation exchange (SCX) followed by reverse phase (RP) chromatography coupled directly in line with electrospray ionization (ESI) ion trap tandem mass spectrometry (2D-LC ESI MS/MS).

Analysis of proteins from uninfected bovine monocytes revealed proteins related to antigen pattern recognition, uptake and presentation to immunocompetent lymphocytes (Lee *et al.* 2006). DDF- MudPIT detected low-abundance cytosolic proteins, indicating the high sensitivity of this approach. Upon comparing proteins from BVDV infected and uninfected monocytes, label free protein quantification methods were used to utilize sampling parameters for estimating protein expression including the sum of cross correlation (ΣXcorr) of identified peptides to enhance the coverage of differentially expressed proteins between the two samples. This approach revealed alterations in the expression of proteins related to immune functions such as cell adhesion, apoptosis, antigen uptake, processing and presentation, acute phase response proteins and MHC class I- and II-related proteins (Lee *et al.* 2009).

In addition, proteomics showed the effects of BVDV biotypes infection on the expression of protein kinases and related proteins involved in the development of viral infection and oncogenic transformation of cells and proteins related to professional antigen presentation. We found that six protein kinases related to cell migration, anti-viral protection, sugar metabolism, and possibly the expression of the receptor for activated C kinase (RAC) were differentially expressed between the ncp and cp BVDV-infected monocytes (Pinchuk *et al.* 2008).

To link the observed differences in protein expression to their broader biological role, information regarding the functions of these gene products and how they interact was

obtained using the Gene Ontology (GO) (Ashburner *et al*. 2000) and systems biology (Aggarwal & Lee 2003), respectively. GO information categorizes functional information for a gene product into three broad categories; identify molecular functions, biological processes and cellular components. A complementary approach, system biology, allows for the exploration and visualization of networks and pathways significantly represented in the proteomics datasets. Identification of altered host proteins by cp or ncp BVDV infection was based on rigorous statistical methods for peptide identification and control of false positive identification (Ammari *et al*. 2010). When followed by GO functional and pathway analysis, this study identified similarities and differences between BVDV biotypes and also showed, as expected, that cp BVDV had a more profound effect on infected monocytes than ncp BVDV. The top under- and over- represented GO functions are shown in figure 3. At Ingenuity Pathways Analysis (IPA; Ingenuity system, California) threshold of significance, 6 and 4 networks and 42 and 33 functions/diseases were significantly represented in the proteomes of ncp and cp BVDV-infected monocytes, respectively. The top ten functions/diseases and signalling pathways (ranked based on significance) are shown here in table 3 (for more details see reference (Ammari *et al*. 2010)). Interestingly, among 69 proteins that have been altered by both biotypes only two proteins, integrin alpha 2b (ITGA2B) and integrin beta 3 (ITGB3), were differentially altered by cp and ncp BVDV biotypes.

Since those studies focus on host immune-related proteins, another general analysis was done to identify the effect of BVDV infection on cells overall protein expression. This showed that the mitochondrial dysfunction pathway was the pathway most affected by cp BVDV, but not by ncp BVDV infection, indicating the induction of apoptosis by cp BVDV. BVDV biotypes differ in their effect on Na+-dependent phosphate transporter (SDPT). SDPT is a transmembrane transporter of inorganic phosphate and examples of cells responding to an increase in the inorganic phosphate by apoptosis are known (Di Marco *et al*. 2008). Its two most prevalent representatives are NaP_i1 and NaP_i2. The up-regulation of SDPT observed in our studies in cells infected by cp BVDV may indicate that this cytopathic virus uses the NaP_i to induce the apoptotic death of the cells that it infects. On the other hand, both cp and ncp BVDV biotypes down-regulated a different isoform of SDPT (NaP_i2b), suggesting that this other isoform of the transporter may not be directly involved in apoptosis. Also, our proteome analysis revealed that two proteins from the mitochondrial voltage-dependent anion channel (VDAC) family, as well as hexokinase (HK) were differentially regulated by cp and ncp BVDV. In addition, cp BVDV-infection contributed to oxidative stress by disturbance of cellular antioxidants system. These data are in line with previous findings about the involvement of these proteins in apoptosis (manuscript in preparation).

Overall, the use of MS-based methods provided a means to study BVDV pathogenesis in a more-high-throughput parallel fashion than individual immunopreciptation studies and this global scale of study increased our ability to assess immune-related protein interaction and cellular changes that are significantly compromised in monocytes infected with BVDV biotypes. Thus, they open new possibilities for discovery of previously unknown viral-host connections. Other proteomics techniques that have been used for other viruses include quantitative methods such as isotype-coded affinity tags (ICAT) and different gel electrophoresis (DIGE) (for a review on the use of proteomics in studying different viruses, see reference (Maxwell & Frappier 2007)).
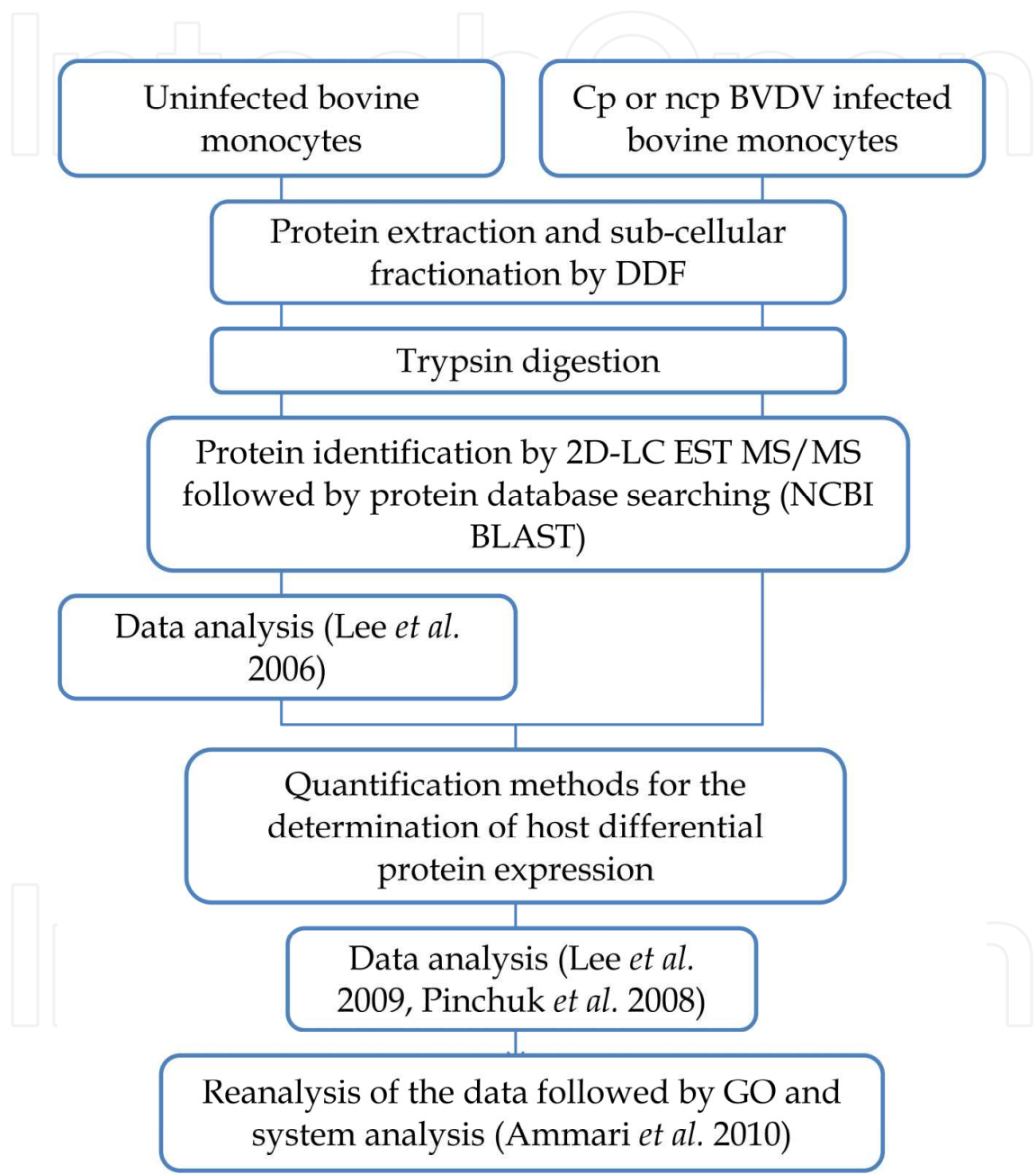
Fig. 2. Flow chart of MS-related work for identifying BVDV-induced changes in bovine monocytes
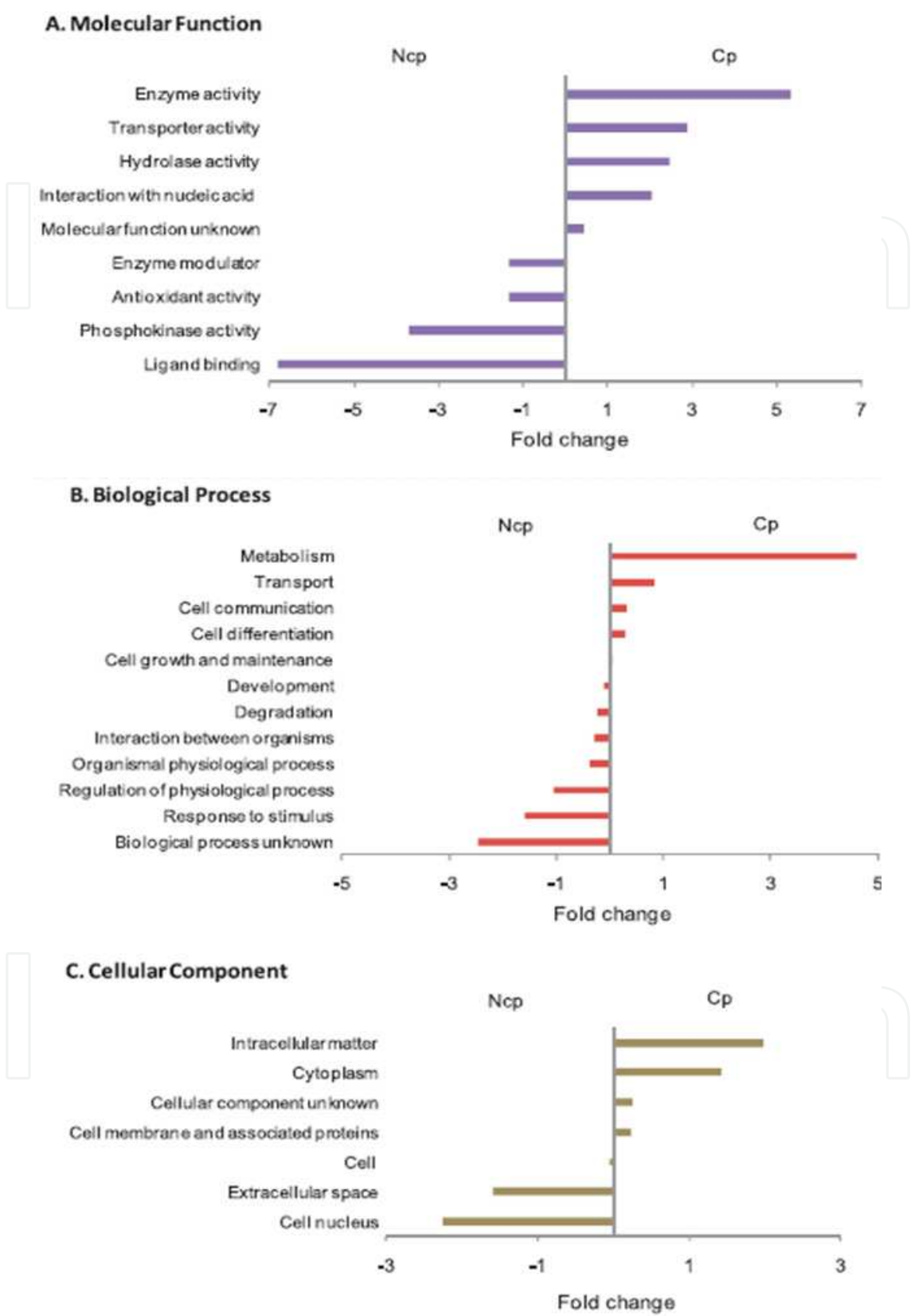
A. Molecular Function

B. Biological Process

C. Cellular Component

Fig. 3. GO functional analysis of host proteins differentially expressed in ncp or cp BVDV-infected monocytes (Ammari *et al*. 2010)

	Cp BVDV-infection	Ncp BVDV-infection
Networks	Four	Six
Pathways	Acute phase response signaling (12), Fcγ receptor mediated phagocytosis in macrophages and monocytes (8), **Actin cytoskeleton signaling (8)**, Antigen presentation pathway (3), B cell development (3), **RhoA signaling (5)**, Caveolae-mediated endocytosis signaling (4), **Clathrin-mediated endocytosis signaling (6)**, IL-10 signaling (3), **Interferon signaling (2)**	Acute phase response signaling (8), Caveolae-mediated endocytosis signaling (5), B cell development (3), IL-10 signaling (3), **Macropinocytosis signaling (3), Virus entry via endocytic pathway (3)**, Antigen presentation pathway (2), Fcγ receptor mediated phagocytosis in macrophages and monocytes (3), **Integrin signaling (4), Primary immunodeficiency signaling (2)**
Functions/ diseases	Cellular function and maintenance (7), cell-to-cell signaling and interaction (8), Inflammatory response (14), **Cellular assembly and organization (2), Protein synthesis (2), Cellular compromise (6), Cell morphology (3)**, Cellular development (4), Hematological system development and function (9), Immune cell trafficking (9)	Cellular movement (10), Immune cell trafficking (18), Inflammatory response (20), **Cell death (11)**, Hematological system development and function (21), Cell-to-cell signaling and interaction (10), Cellular function and maintenance (5), **Immunological disease (5), Antigen presentation (13), Inflammatory disease (5)**

Table 3. Top ten functions/diseases and pathways in BVDV- infected monocytes. Numbers between brackets indicates the number of altered proteins involved in the function/disease or pathway

5. Concluding remarks

By using proteomics it is possible now to separate very complex protein mixtures with high resolution, to extract the proteins of interest, to study them with MS and to identify them with high reliability. With the use of new proteomics approaches, much can be done in studying BVDV protein composition, structure, and interactions. We expect that the application of proteomic methods to study BVDV will provide valuable information about BVDV pathogenesis and reveal new insights about host-virus interactions, leading to better strategies to prevent or cure BVDV infection. However, one should keep in mind that proteomics should always be considered the starting point for functional studies rather than an end point that follows traditional methods.

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Proteomic Applications in Biology

Edited by Dr. Joshua Heazlewood

The past decade has seen the field of proteomics expand from a highly technical endeavor to a widely utilized technique. The objective of this book is to highlight the ways in which proteomics is currently being employed to address issues in the biological sciences. Although there have been significant advances in techniques involving the utilization of proteomics in biology, fundamental approaches involving basic sample visualization and protein identification still represent the principle techniques used by the vast majority of researchers to solve problems in biology. The work presented in this book extends from overviews of proteomics in specific biological subject areas to novel studies that have employed a proteomics-based approach. Collectively they demonstrate the power of established and developing proteomic techniques to characterize complex biological systems.

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