The effect of infectious bursal disease virus (IBDV) infection on cellular protein expression is essential for viral pathogenesis. To characterize the cellular response to IBDV infection, the differential proteomes of chicken embryo fibroblasts, with and without IBDV infection, were analyzed at different time points with two-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF/TOF identification. Comparative analysis of multiple 2-DE gels revealed that the majority of protein expression changes appeared at 48 and 96 h after IBDV infection. Mass spectrometry identified 51 altered cellular proteins, including 13 up-regulated proteins and 38 down-regulated proteins 12–96 h after infection. Notably 2-DE analysis revealed that IBDV infection induced the increased expression of polyubiquitin, apolipoprotein A-I, heat shock 27-kDa protein 1, actins, tubulins, eukaryotic translation initiation factor 4A isoform 2, acidic ribosomal phosphoprotein, and ribosomal protein S1A isoform 2. In addition, IBDV infection considerably suppressed those cellular proteins involved in ubiquitin-mediated protein degradation, energy metabolism, intermediate filaments, host translational apparatus, and signal transduction. Moreover 38 corresponding genes of the differentially expressed proteins were quantitated by real-time RT-PCR to examine the transcriptional profiles between infected and uninfected chicken embryo fibroblasts. Western blot further confirmed the inhibition of Rho protein GDP dissociation inhibitor expression and the induction of polyubiquitin during IBDV infection. Subcellular distribution analysis of the cytoskeletal proteins vimentin and β-tubulin clearly demonstrated that IBDV infection induced the disruption of the vimentin network and microtubules late in IBDV infection. Thus, this work effectively provides useful dynamic protein-related information to facilitate further investigation of the underlying mechanism of IBDV infection and pathogenesis.

Molecular & Cellular Proteomics 7:612–625, 2008.

From the ‡Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou 310029, China, §State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University, Hangzhou 310003, China, and ¶Key Laboratory of conservation genetics and reproductive biology for endangered wild animals of the Ministry of Education, Zhejiang University, Hangzhou 310058, China

Received, August 20, 2007
Published, MCP Papers in Press, December 4, 2007, DOI 10.1074/mcp.M700396-MCP200

This is an Open Access article under the CC BY license.

1 The abbreviations used are: IBDV, infectious bursal disease virus; 2-DE, two-dimensional gel electrophoresis; CEF, chicken embryo fibroblast; p.i., postinfection; mAb, monoclonal antibody; IFA, indirect immunofluorescence assay; UPP, ubiquitin-proteasome pathway; IF, intermediate filament; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; FHV, flock house virus; TCID50, 50% tissue culture infective dose; GDI, GDP dissociation inhibitor; TRITC, tetramethylrhodamine isothiocyanate; AKR, aldo-keto reductase; ALDOC, aldolase C; HARS, histidyl-tRNA synthetase; RPSA, ribosomal protein SA isoform 2; HNRPLD, heterogeneous nuclear ribonucleoprotein D-like; FPS, farnesyl-diphosphate synthase; MTA, methyltransferase 24; LDHB, lactate dehydrogenase B; CKB, B-creatine kinase.
used proteomics approaches to reveal the effects of viral infection on the cellular proteome (15). The comparative proteomics approaches coupling 2-DE and MS (2-DE/MS) effectively help the study of the molecular profiles of virus-infected cells (16). In plant viruses, 2-DE/MS has been used to study the cellular changes in rice yellow mottle virus-infected cells in susceptible and partially resistant rice cultivars (17) and in tobacco mosaic virus-infected tomato (18). In animal viruses, 2-DE/MS has been utilized to investigate the cellular changes in Vero cells infected with African swine fever virus (19), rh-adomyosarcoma cells infected with enterovirus 71 (20), nuclear proteome changes in A549 alveolar type II-like epithelial cells infected with respiratory syncytial virus (21), and expression changes in B cells infected with Epstein-Barr virus or with Epstein-Barr virus nuclear antigen EBNA-2 (22). In addition, several studies have used distinct protein separation methods, including DIGE, ICATs, or multidimensional liquid chromatographic separations followed by LC-MS/MS identification to investigate the cellular response to infection by severe acute respiratory syndrome-associated coronavirus (SARS-CoV), flock house virus (FHV), and hepatitis C virus (23–25). Therefore, the proteomics strategies provide an overall understanding of the cellular factors involved in various stages of infection and give an insight into the alteration of signaling pathways to further understand viral pathogenesis.

In this study, we utilized a 2-DE/MS proteomics approach followed by Western blot coupled with real time RT-PCR to observe the differentially expressed protein profiles between IBDV-infected and mock-infected CEFs at different time points after infection. A total of 81 differentially expressed protein spots were identified. Further analysis of these data provides clues to understanding the replication and pathogenesis of IBDV and the virus-host interactions.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Virus Infection, and Sample Preparation**—A primary CEF monolayer was prepared from 10-day-old embryonated specific-pathogen-free chicken eggs (Beijing Merial Vital Laboratory Animal Technology Co. Ltd, Beijing, China) and maintained in Hanks’ medium supplemented with 8% fetal bovine serum. The CEF monolayer was inoculated with CEF-adapted IBDV strain NB (10<sup>5</sup> TCID<sub>50</sub>/0.2 ml) (26) with medium-treated CEF monolayer acting as control. At 12, 48, and 96 h postinfection (p.i.), the IBDV-infected and mock-infected cells were mechanically scraped into the culture medium and harvested by centrifugation at 8000 × g for 5 min. The cells were washed three times with ice-cold PBS and lysed with lysis buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 65 mM DTT, 0.2% Bio-Lyte 3/10, and 1 mM PMSF at a volume ratio of 1:10 for 2 h on ice. DNase I (TaKaRa, Shiga, Japan) and RNase A (TaKaRa) at a final concentration of 20 units/ml and 0.25 mg/ml, respectively, were added to degrade nucleic acids on ice for 45 min, and the lysates were then clarified by centrifugation at 20,000 × g at 4 °C for 60 min. The supernatants were collected and stored in single use aliquots at −80 °C. All protein samples were diluted 1:10, and the concentration was determined by a microplate modification of the Bradford assay (27) with 0.5 mg/ml BSA as the standard solution and homogenization buffer as the blank.

**Two-dimensional Gel Electrophoresis and Image Analysis**—We performed a 2-DE analysis using 24-cm ReadyStrip IPG strips (linear, pl 5–8, Bio-Rad) in a PROTEAN IEF Cell and PROTEAN plus Dodeca Cell (Bio-Rad) according to the manufacturer’s instructions. To minimize the analytical variation between gels, the 2-DE conditions suitable for the analysis of 12 gels were optimized by dividing 12 independent samples into three independent sections for IEF under the same conditions and then simultaneously applying the 12 isoelectric-focused samples to SDS-PAGE. The IPG strips were rehydrated at 20 °C for 14 h with 450 µl of rehydration buffer (7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 65 mM DTT, and 0.2% Bio-Lyte 3/10) containing 250 µg of protein. The rehydrated strips were automatically focused using the following parameters: 250 V, linear, 1 h; 1000 V, linear, 1 h; 10,000 V, linear, 5 h; 10,000 V, rapid, 90,000 V-h. The isoelectric-focused strips were incubated for 15 min in an equilibration buffer (6 M urea, 30% glycerol, 2% SDS, and 0.375 M Tris, pH 8.8) containing 1% DTT and then incubated for 15 min in an equilibration buffer containing 2.5% iodoacetamide. The equilibrated IPG strips were further resolved by 11% SDS-PAGE at 80 V for 45 min and then 200 V until the dye front reached the bottom of the gels. The gels were stained by the modified silver staining method compatible with MS (28) and scanned at a resolution of 500 dots/inch using the Uniscan D3000 scanner (Tsinghua, Beijing, China). Spot detection, spot matching, and quantitative intensity analysis were performed using PDQuest 2-D analysis software (Bio-Rad). The gel images were normalized according to the total quantity in the analysis set. Relative comparison of intensity abundance between IBDV-infected and mock-infected groups at three time points (three replicate samples for each group) was performed using Student’s t test. Expression intensity ratio<sub>infected/uninfected</sub> values larger than 2.0 (p < 0.05) or smaller than 0.5 (p ≤ 0.05) were set as a threshold indicating significant changes (29).

**In-gel Tryptic Digest**—The protein spots were manually excised from the silver-stained gels and then transferred to V-bottom 96-well microplates loaded with 100 µl of 50% ACN, 25 mM ammonium bicarbonate solution/well. After being destained with 1 h, gel plugs were dehydrated with 100 µl of 100% ACN for 20 min and then thoroughly dried in a SpeedVac concentrator (Thermo Savant) for 30 min. The dried gel particles were rehydrated at 4 °C for 45 min with 2 µl/well trypsin (Promega, Madison, WI) in 25 mM ammonium bicarbonate and then incubated at 37 °C for 12 h. After trypsin digestion, the peptide mixtures were extracted with 8 µl of extraction solution (50% ACN, 0.5% TFA)/well at 37 °C for 1 h. Finally the extracts were dried under the protection of N<sub>2</sub>.

**MALDI-TOF/TOF MS and MS/MS Analysis and Database Search**—The peptide mixtures were redissolved in 0.8 µl of matrix solution (α-cyano-4-hydroxycinnamic acid (Sigma) in 0.1% TFA, 50% ACN) and then spotted on the MALDI plate. Samples were allowed to air dry and analyzed by a 4700 MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA). Trypsin-digested peptides of myoglobin were added to the six calibration spots on the MALDI plate to calibrate the mass instrument with internal calibration mode. The UV laser was operated at a 200-Hz repetition rate with wavelength of 355 nm. The accelerated voltage was operated at 20 kV. All acquired spectra of samples were processed using 4700 Explore® software (Applied Biosystems) in a default mode. Parent mass peaks with mass range of 700–3200 Da and minimum signal to noise ratio of 20 were picked out for tandem TOF/TOF analysis. Combined MS and MS/MS spectra were submitted to MASCOT (Version 2.1, Matrix Science, London, UK) by GPS Explorer software (Version 3.6, Applied Biosystems) and searched with the following parameters: National Center for Biotechnology Information non-redundant (NCBI_nr) database (release date, March 18, 2006), taxonomy of bony vertebrates or viruses, trypsin digest with one missing cleavage, no fixed modifications, MS tolerance of 0.2 Da, MS/MS tolerance of 0.6 Da, and possible oxidation of methionine.
Known contaminant ions (human keratin and tryptic autodigest peptides) were excluded. A total of 4,736,044 sequences and 1,634,373,987 residues in the database were actually searched. MASCOT protein scores (based on combined MS and MS/MS spectra) of greater than 72 were considered statistically significant (p ≤ 0.05). The individual MS/MS spectrum with a statistically significant (confidence interval ≥ 95%) ion score (based on MS/MS spectra) were accepted. To eliminate the redundancy of proteins that appeared in the database under different names and accession numbers, the single protein member belonging to the species Gallus or else with the highest protein score (top rank) was singled out from the multiprotein family.

**Real Time RT-PCR**—Specific primers (supplemental Table 1) suited to simultaneously amplify various target genes were designed according to the corresponding gene sequences of MS/MS-identified proteins and the available gene information deposited in the GenBank library by using the Lasergene sequence analysis software (DNASTAR, Inc., Madison, WI). The CEF monolayers inoculated with IBDV strain NB for 12, 24, and 48 h were washed three times with ice-cold PBS and then lysed with TRizol reagent (Invitrogen). Total cellular RNA was extracted using the RNeasy minikit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer’s protocol. RNA concentrations were measured using a spectrophotometer (260/280 nm). After heating at 65 °C for 5 min to denature RNA and to inactivate RNases, 1 μg of total RNA was subjected to reverse transcription using 200 units of SuperScript III reverse transcriptase (Invitrogen), 40 units of RNaseOUT recombinant RNase inhibitor (Invitrogen), 200 ng of random hexamer primers (TaKaRa), 0.5 mm (each) dNTPs (TaKaRa), 4 μl of 5× First-Strand Buffer (Invitrogen), and 1 μl of 0.1 M DTT (Invitrogen) in a total volume of 20 μl at 37 °C for 5 min and then incubated at 50 °C for 1 h. The reaction was terminated by heating at 70 °C for 15 min. The real time RT-PCR was performed by using the 7500 Real-Time PCR System (Applied Biosystems) in a total volume of 20 μl containing 100 ng of cDNA template, 1× SYBR Premix Ex Taq (Perfect Real time PCR, TaKaRa), and a 200 mM concentration of each primer. After initial denaturation at 95 °C for 30 s, the amplification was carried out through 40 cycles, each consisting of denaturation at 95 °C for 15 s, primer annealing at 58 °C for 15 s, and DNA extension at 72 °C for 40 s. Melting curves were obtained, and quantitative analysis of the data was performed using the 7500 System SDS software Version 1.3.1 in a relative quantification (dCtCt) study model (Applied Biosystems). Parallel mock-infected CEFs were used as controls.

**Western Blot and Densitometric Analysis**—Samples of IBDV-infected and uninfected CEFs were lysed at different time points p.i., and the protein concentrations were determined as described above. Equivalent amounts of cell lysates (70 μg) were subjected to 12% SDS-PAGE and then transferred to nitrocellulose cellulose membranes (Hybond-C extra, Amersham Biosciences). After blocking, the membranes were incubated with mouse monoclonal antibodies (mAbs) to β-tubulin (Physarum polycephalum myxamoebae) (clone KMX-1, Chemicon), swine vimentin (clone V9, Chemicon), bovine ubiquitin (clone P4D1, Cell Signaling Technology, Boston, MA), and human Rho-GDI (clone 16, BD Biosciences), respectively. The membranes were then incubated with horse radish peroxidase-conjugated anti-mouse IgG (Kirkegaard & Perry Laboratories, Inc.) and visualized using 3,3′,5,5′-tetramethylbenzidine-stabilized substrate (Promega). Finally the immunoblots were scanned, and densitometric analysis was performed using the Bio-Rad Quantity One software. Each reaction was performed in triplicate.

**Double Staining Immunofluorescence and Nuclear Staining**—CEF and Vero cells inoculated with the virulent IBDV were cultured for 24 and 60 h, respectively. The cells were washed twice with PBS and fixed with cold acetone/methanol (1:1) for 20 min at −20 °C and then allowed to air dry. The fixed cells were incubated with a mixture of rabbit antiserum to IBDV-VP1 prepared in our laboratory (30) and the above mentioned mAbs (β-tubulin, vimentin, ubiquitin, and Rho-GDI) for 90 min at 37 °C in a humidified chamber. After washing thrice with PBS, the cells were incubated for 60 min at 37 °C with a mixture of TRITC-conjugated goat anti-rabbit IgG (Sigma) and FITC-conjugated goat anti-mouse IgG (Kirkegaard & Perry Laboratories, Inc.). The additional nuclear staining with 4′,6-diamidino-2-phenylindole (Sigma) was performed as described previously (5). The triple stained cells were washed thrice with PBS and subsequently examined under a Zeiss LSM510 laser confocal microscope.

**RESULTS**

**Two-dimensional Gel Electrophoresis Profiles of IBDV-infected CEFs**—To obtain a detailed comparison of the differences in protein expression profile, the cellular proteins of IBDV-infected and mock-infected CEFs were extracted for 2-DE analysis at 12, 48, and 96 h p.i. In the 2-DE analysis, the spots of detectable protein ranged from 1400 to 1650 spot on 24-cm two-dimensional gels (pl 5–8) loaded with 250 μg of total cellular proteins/gel. In the IBDV-infected cells, no obvious changes were observed in the numbers of spots of detectable protein at 12 h p.i., but the numbers of spots of expressed protein decreased gradually from 48 to 96 h p.i. (Fig. 1A). On the basis of the average intensity ratios of protein spots, a total of 102 protein spots were found to be dynamically changed in IBDV-infected CEFs, including 33 significantly up-regulated protein spots (ratio_{infection/mock} ≥ 2, p ≤ 0.05; Fig. 1B) and 69 significantly down-regulated protein spots (ratio_{infection/mock} ≤ 0.5, p ≤ 0.05; Fig. 1C). As shown in Table I and supplemental Table 2, of the up-regulated proteins in infected CEFs, three protein spots showed signs of up-regulation at 12 h p.i., 28 protein spots were up-regulated at 48 h p.i., and two protein spots were newly induced at 96 h p.i. Among the down-regulated proteins, five protein spots displayed complete inhibition at 48 and 96 h p.i., and the remainder showed signs of a decrease at 48 and 96 h p.i. In general, the majority of protein expression changes appeared at 48 and 96 h after IBDV infection.

**Mass Spectral Identification of Differentially Expressed Proteins**—To identify the differentially expressed proteins in IBDV-infected CEFs in 2-DE gels, a total of 102 protein spots with a threshold greater than 2-fold were excised from these 2-DE gels and subjected to in-gel trypsin digestion and subsequent MALDI-TOF/TOF identification. As shown in Table I and Fig. 2, 81 differentially expressed spots were successfully identified (the MS and MS/MS spectra are listed in the supplemental figure). According to annotations from the UniProt Knowledgebase (Swiss-Prot/TrEMBL) and Gene Ontology Database, the identified cellular proteins were involved in the cytoskeleton, in stress response, in macromolecular biosynthesis, in ubiquitin-proteasome pathway (UPP), in signal transduction, and in metabolic enzymes. The percentage of up-regulated and down-regulated protein spots was 26.92 and 73.08%, respectively. Thirty-one up-regulated spots corresponded to the following 15 proteins: two viral proteins...
(34%), seven cytoskeletal proteins (48%), three macromolecular biosynthesis proteins (9%), and three other cellular proteins (9%) (Fig. 3A). ACT5 and polyubiquitin, in particular, were expressed in IBDV-infected cells as newly induced proteins. These up-regulated cellular proteins were located mainly in the cytoplasm, including cytoskeleton (47%), cytosol (38%), and ribosome (9%) (Fig. 3B).

The 50 down-regulated spots were found to correspond to 38 cellular proteins. These proteins were mainly involved in UPP (17%), signal transduction (17%), carbohydrate degradation (16%), intermediate filament (IF) proteins (14%), and other functions (Fig. 3C). Interestingly all of the proteins involved in signal transduction, metabolism, and biosynthesis were down-regulated during IBDV infection. In particular, CAPZA1, AHA1, UCHL5, and Rho-GDI were inhibited in the late stages of IBDV infection. These down-regulated protein spots were mainly distributed within the cytoplasm, including cytosol (34%), cytoskeleton (24%), and cell organelles (e.g. endoplasmic reticulum, ribosome, and mitochondrion); in addition, some proteins were located in the nucleus and membrane (Fig. 3D). In this result, many different spots were identified to be the products of the same gene, including IBDV viral proteins, ACTB, ACT5, vimentin, CAPZA1, HSPB1, AKR, and ALDOC. Surprisingly there were two spots, one up-regulated and the other down-regulated, that were both identified as HSPB1.

**Transcriptional Profiles of Differentially Expressed Proteins during IBDV Infection**—Approximately 50% of the IBDV-infected CEF monolayer with cytopathic effect detached from the cell bottle at 48 h p.i. As a result, the transcriptional alterations of 38 selected genes of CEF monolayers with and without IBDV infection were analyzed at 12, 24, and 40 h p.i. by using the mRNA transcript of the histone H5 gene as a control housekeeping gene. Changes in relative expression levels greater than 2-fold (ddCT/H11350, p/H11349 < 0.05) were considered to be significant. In general, the trends in the change in mRNA abundance of the 31 genes at three time points p.i. were similar to the change patterns of their corresponding proteins in 2-DE gels (Fig. 4). Of these genes, the mRNA abundance of genes ACTA, HARS, MTA, and PSME3 shared a persistent decrease during 12–40 h p.i., and 28 gene transcripts represented a marked elevation at 12 h after IBDV infection and a persistent decrease between 24 h and 40 h p.i. However, the transcripts of genes VIM, HSPB1, and RPSA were down-regulated at 12 h p.i. and up-regulated between 24 and 40 h p.i. The transcriptional pattern of the FPS gene was down-regulated at 12 and 40 h p.i. and elevated at 24 h p.i. Conversely the transcription of ARPP0 and EN01 genes exhibited an up-regulation at 12 and 40 h p.i. and a down-regulation at 24 h p.i. These data provide transcriptional information complementary to the differentially expressed proteins detected by proteomics analysis.

**Western Blot Validation**—To further confirm the dynamic alterations of protein expression during IBDV infection, the four proteins ubiquitin, Rho-GDI, vimentin, and β-tubulin were selected for Western blot analysis. Equal amounts of cell
### Table I

List of the differentially expressed protein spots in IBDV-infected CEFs identified by MALDI-TOF or MALDI-TOF/TOF

| Spot no. | Protein name Abbr. | Accession No. | Mr (pred/exp) | pl (pred/exp) | Mateched/unmatched | Abundance Ratio<sub>infected/uninfected</sub> (Means±SD) | Cov era (%) | Protein score / best ion score | Peptides Identified |
|----------|-------------------|---------------|----------------|----------------|---------------------|---------------------------------|----------|--------------------------------|---------------------|
| 1127 VP3 | VP3               | gi|14715597     | 109.6/29.6     | 5.7/5.55        | 6/2                 | NI** NI*                        | 4.9      | 0.0 125/49                     | YGTAGYGVEAR         |
| 4121 VP3 | VP3               | gi|2597439      | 32.74/30.85    | 6.75/6.44       | 9/34               | NI** NI*                        | 28.3     | 0.0 182/87                     | YGTAGYGVEAR         |
| 2119 VP3 | VP3               | gi|14582976     | 107.26/29.48   | 5.93/5.92       | 6/3                 | NI** NI*                        | 7.6      | 109/33                         |                    |
| 5120 VP3 | VP3               | gi|832973       | 110.49/30.96   | 5.81/6.75       | 8/20               | NI* NI*                         | 9.5      | 238/74                         |                    |
| 1022 VP4 | VP4               | gi|2160353      | 107.47/28.45   | 6.05/5.67       | 11/18              | NI* NI*                         | 16.1     | 310/83                         |                    |
| 1023 VP4 | VP4               | gi|1854394      | 72.78/27.89    | 6/5.68          | 5/5                 | NI** NI*                        | 6.9      | 121/37                         |                    |
| 2019 VP4 | VP4               | gi|2160353      | 107.47/28.28   | 6.05/6.04       | 9/7                 | NI*** NI*                       | 13.4     | 311/87                         |                    |
| 3118 VP4 | VP4               | gi|2160353      | 107.47/28.82   | 6.05/6.33       | 8/2                 | NI* NI*                         | 12.7     | 263/77                         |                    |
| 3119 VP4 | VP4               | gi|2160353      | 107.47/29.29   | 6.05/6.33       | 6/1                 | NI* NI*                         | 7.4      | 219/88                         |                    |
| 4031 VP4 | VP4               | gi|89145882     | 109.68/28.69   | 6.05/6.59       | 10/19               | NI* NI*                         | 11.7     | 187/58                         |                    |
| 4133 VP4 | VP4               | gi|2160353      | 107.47/28.89   | 6.05/6.47       | 6/1                 | NI** NI*                        | 8        | 266/75                         |                    |

### Viral proteins of IBDV

| Protein | Accession No. | Mr (pred/exp) | pl (pred/exp) | Mateched/unmatched | Abundance Ratio<sub>infected/uninfected</sub> (Means±SD) | Cov era (%) | Protein score / best ion score | Peptides Identified |
|---------|---------------|---------------|---------------|---------------------|---------------------------------|----------|--------------------------------|---------------------|
| VP3     | gi|14715597     | 109.6/29.6     | 5.7/5.55        | 6/2                 | NI** NI*                        | 4.9      | 0.0 125/49                     | YGTAGYGVEAR         |
| VP3     | gi|2597439      | 32.74/30.85    | 6.75/6.44       | 9/34               | NI** NI*                        | 28.3     | 0.0 182/87                     | YGTAGYGVEAR         |
| VP3     | gi|14582976     | 107.26/29.48   | 5.93/5.92       | 6/3                 | NI** NI*                        | 7.6      | 109/33                         |                    |
| VP3     | gi|832973       | 110.49/30.96   | 5.81/6.75       | 8/20               | NI* NI*                         | 9.5      | 238/74                         |                    |
| VP4     | gi|2160353      | 107.47/28.45   | 6.05/5.67       | 11/18              | NI* NI*                         | 16.1     | 310/83                         |                    |
| VP4     | gi|1854394      | 72.78/27.89    | 6/5.68          | 5/5                 | NI** NI*                        | 6.9      | 121/37                         |                    |
| VP4     | gi|2160353      | 107.47/28.28   | 6.05/6.04       | 9/7                 | NI*** NI*                       | 13.4     | 311/87                         |                    |
| VP4     | gi|2160353      | 107.47/28.82   | 6.05/6.33       | 8/2                 | NI* NI*                         | 12.7     | 263/77                         |                    |
| VP4     | gi|2160353      | 107.47/29.29   | 6.05/6.33       | 6/1                 | NI* NI*                         | 7.4      | 219/88                         |                    |
| VP4     | gi|89145882     | 109.68/28.69   | 6.05/6.59       | 10/19               | NI* NI*                         | 11.7     | 187/58                         |                    |
| VP4     | gi|2160353      | 107.47/28.89   | 6.05/6.47       | 6/1                 | NI** NI*                        | 8        | 266/75                         |                    |

### Cytoskeleton proteins

| Protein | Accession No. | Mr (pred/exp) | pl (pred/exp) | Mateched/unmatched | Abundance Ratio<sub>infected/uninfected</sub> (Means±SD) | Cov era (%) | Protein score / best ion score | Peptides Identified |
|---------|---------------|---------------|---------------|---------------------|---------------------------------|----------|--------------------------------|---------------------|
| α-actin | ACTA          | gi|8489855      | 41.89/39.96    | 5.18/6.67        | 3/3                 | 1.24±0.02 7.47±0.17** 6.51±0.68* | 14.6     | 84/28  | QYEDESQPSIVHR SYELPDGQVITIGNER |
| β-actin | ACTB          | gi|63018        | 41.74/41.14    | 5.29/6.68        | 9/10               | 1.28±0.02 3.53±0.27* 3.68±0.13* | 31.2     | 266/66 | QYEDESQPSIVHR SYELPDGQVITIGNER |
| β-actin | ACTB          | gi|63018        | 41.74/39.22    | 5.29/6.69        | 8/11               | 1.45±0.18 4.54±0.28* 2.84±0.34* | 31.5     | 407/87 | QYEDESQPSIVHR SYELPDGQVITIGNER |
| β-actin | ACTB          | gi|63018        | 41.74/40.71    | 5.47/6.84        | 10/14              | 1.01±0.16 5.51±0.16** 6.52±0.47* | 33.3     | 330/83 | QYEDESQPSIVHR SYELPDGQVITIGNER |
| β-actin | ACTB          | gi|63018        | 41.74/39.33    | 5.29/6.86        | 6/20               | 0.97±0.20 2.97±0.52** 2.26±0.16** | 22.7     | 293/78 | QYEDESQPSIVHR SYELPDGQVITIGNER |
| Protein Name      | Accession | GI       | PI          | PD          | MW          | pI          | SD          | Cell Type       |
|-------------------|-----------|----------|-------------|-------------|-------------|-------------|-------------|----------------|
| Actin, cytoplasmic type 5 | ACT5 gi|56119084  | 41.81/34.2  | 5.29/7.25   | 6/3         | 0.73±0.04  | 9.85±2.25** | 16.45±2.48*  | 16.1 28.4 QEYDESGPSIVHR SYELPDQVITIGNER VAPEHPVLLTEAPLN PK |
| Actin, cytoplasmic type 5 | ACT5 gi|56119084  | 41.81/23.5  | 5.3/7.31    | 2/25        |             |             | 15.2 263/79   | 89/31 |
| Actin, cytoplasmic type 5 | ACT5 gi|1703121   | 40.07/31.9  | 5.3/7.4/3   | 3/0         |             |             | 13.3 142/65   | 93/24 |
| Capping protein muscle Z-line alpha 1 | CAPZ gi|45382905  | 32.9/35.91  | 5.43/7.11   | 7/12        | 0.92±0.01  | 0.34±0.03*  | ND* 36.7 147/23 |
| Capping protein muscle Z-line alpha 1 isoform 3 | CAPZ gi|73981498  | 5.91/36.54  | 4.54/7.11   | 2/3         | 1.75±0.12  | 0.42±0.09*  | 0.48±0.06*  | 53.8 72/22 |
| Calponin 3 | CNN3 gi|50751284  | 36.59/38.54  | 6.14/6.07   | 5/4         | 1.26±0.08  | 0.33±0.05*  | 0.20±0.08** | 15.1 135/59 DHYHQYQSDQGIDY |
| Vimentin | VIM gi|50732409  | 53.12/51.75  | 5.24/7.31   | 32/16       | 1.25±0.13  | 0.43±0.03*  | 0.13±0.01*  | 77.1 513/76 LGDLYEEMR INMIPITFASLNLR LDQDEIQNMKEEMAR |
| Vimentin | VIM gi|50732409  | 53.12/51.75  | 5.24/7.41   | 30/14       | 1.12±0.22  | 0.49±0.09** | 0.39±0.01** | 69.2 441/59 LGDLYEEMR |
| Vimentin | VIM gi|50732409  | 53.12/53.53  | 5.24/7.50   | 24/6        | 1.12±0.35  | 0.46±0.09** | 0.28±0.01*  | 59.2 546/146 INMIPITFASLNLR LDQDEIQNMKEEMAR EMEENFAEANYQ DTIGR |
| Vimentin | VIM gi|50732409  | 53.12/53.13  | 5.24/7.61   | 30/3        | 1.15±0.11  | 0.55±0.08*  | 0.28±0.13*  | 75.8 513/61 FADLSEAANR LGDLYEEMR |

**Proteomic Dynamic Analysis of IBDV-infected Host Cells**

Molecular & Cellular Proteomics 7.3 617
### Table I—continued

| Entry     | Symbol |gi| Protein Name                                                                 | Accession | Mw [kDa] | pI | %ID | %CARB | %CARB | %Carb | %Carb | MAF | MAF  |
|-----------|--------|---|--------------------------------------------------------------------------------|-----------|---------|----|-----|-------|-------|-------|-------|-----|------|
| 8318      | vimentin |gi|50732409 | 53.12/46.9 | 5.24/7.69 | 15/6 | 1.01±0.03 | 0.47±0.04 | 0.25±0.03* | 35 | 270/40 |
| 8415      | vimentin |gi|50732409 | 53.12/49.8 | 5.24/7.75 | 25/5 | 0.96±0.04 | 0.69±0.13* | 0.16±0.03* | 58 | 610/102 |
| 7620      | chicken lamin B2 |gi|63560 | 67.90/66.87 | 5.31/7.30 | 26/2 | 1.11±0.26 | 0.46±0.06** | 0.41±0.08* | 46.3 | 450/62 |

**Stress response**

| Entry     | Symbol |gi| Protein Name                                                                 | Accession | Mw [kDa] | pI | %ID | %CARB | %CARB | %Carb | %Carb | MAF | MAF  |
|-----------|--------|---|--------------------------------------------------------------------------------|-----------|---------|----|-----|-------|-------|-------|-------|-----|------|
| 5030      | heat shock 27 kDa protein 1 |HSPB1 |gi|45384222 | 21.66/24.99 | 5.77/6.75 | 4/5 | NI** | NI* | 31.6 | 182/83 |
| 4013      | heat shock 27 kDa protein 1 |HSPB1 |gi|45384222 | 21.66/25.27 | 5.77/6.61 | 3/4 | 0.91±0.03 | 0.40±0.10* | 0.32±0.05* | 23.3 | 147/39 |
| 5212 AHA1 activator of heat shock 90 kDa protein ATPase homolog 1 |AHA1 |gi|50748536 | 38.02/40.75 | 5.67/6.78 | 9/13 | 23.3 | 123/16 |

**Macromolecular biosynthesis**

| Entry     | Symbol |gi| Protein Name                                                                 | Accession | Mw [kDa] | pI | %ID | %CARB | %CARB | %Carb | %Carb | MAF | MAF  |
|-----------|--------|---|--------------------------------------------------------------------------------|-----------|---------|----|-----|-------|-------|-------|-------|-----|------|
| 4112      | acidic ribosomal phosphoprotein ARPP0 |gi|45384494 | 34.26/36.32 | 5.72/6.55 | 9/14 | 1.29±0.08 | 0.78±0.37** | 27.5 | 154/68 |
| 5105      | ribosomal protein SA isoform 2 |PSA |gi|50732898 | 33.00/29.58 | 4.86/8.60 | 4/7 | 1.49±0.18 | 6.00±0.20** | 5.72±0.20* | 17.9 | 230/76 |
| 5303 eukaryotic translation initiation factor 4A, isoform 2 |EIF4A2 |gi|45383077 | 46.34/41.78 | 5.33/6.77 | 11/2 | 7.32±0.25** | 66.1±0.06* | 6.13±0.10** | 15.3 | 264/73 |
| 7211      | eukaryotic translation initiation factor 2, subunit 1 alpha |EIF2S1 |gi|57529969 | 33.04/37.57 | 6.09/7.44 | 14/5 | 0.91±0.03 | 0.40±0.10* | 0.32±0.05* | 23.3 | 123/16 |
| 5402      | histidyl-tRNA synthetase |HARS |gi|50750103 | 30.30/29.77 | 6.46/6.46 | 4/3 | 1.21±0.12 | 0.53±0.04* | 0.28±0.06* | 57.3 | 292/41 |

**RNA processing and biosynthesis**

| Entry     | Symbol |gi| Protein Name                                                                 | Accession | Mw [kDa] | pI | %ID | %CARB | %CARB | %Carb | %Carb | MAF | MAF  |
|-----------|--------|---|--------------------------------------------------------------------------------|-----------|---------|----|-----|-------|-------|-------|-------|-----|------|
| 1213      | heterogeneous nuclear ribonucleoprotein D-like |HNRPD |gi|71896741 | 33.42/37.07 | 6.09/7.44 | 14/5 | 0.91±0.03 | 0.40±0.10* | 0.32±0.05* | 23.3 | 123/16 |
| 5105      | polyubiquitin |UBI |gi|50732409 | 53.12/46.9 | 5.24/7.69 | 15/6 | 1.01±0.03 | 0.47±0.04 | 0.25±0.03* | 35 | 270/40 |
| 8415      | vimentin |VIM |gi|50732409 | 53.12/49.8 | 5.24/7.75 | 25/5 | 0.96±0.04 | 0.69±0.13* | 0.16±0.03* | 58 | 610/102 |

**Ubiquitin-proteasome pathway**

| Entry     | Symbol |gi| Protein Name                                                                 | Accession | Mw [kDa] | pI | %ID | %CARB | %CARB | %Carb | %Carb | MAF | MAF  |
|-----------|--------|---|--------------------------------------------------------------------------------|-----------|---------|----|-----|-------|-------|-------|-------|-----|------|
| 2219      | polyubiquitin |UBI |gi|3954791 | 17.74/38.27 | 5.92/5.94 | 3/10 | NI* | NI* | 19.7 | 82.39 |
| 0012      | proteasome subunit beta-type |PSM1 |gi|3680347 | 52.57/7.39 | 3/18 | 1.50±0.15 | 0.61±0.09* | 0.28±0.06* | 26.3 | 106/86 |
| 0013      | proteasome (prosome, macropain) subunit, alpha type 2 |PSMA2 |gi|61098039 | 9.13/25.50 | 9.26/5.48 | 3/9 | 0.95±0.09 | 0.38±0.01* | 0.34±0.07** | 41.2 | 75/36 |
| 0308      | proteasomal ATPase (SUG1) |PSMD5 |gi|50750616 | 46.14/44.50 | 7.11/5.34 | 4/7 | 1.08±0.13 | 0.52±0.05** | 0.50±0.13** | 10.3 | 83/27 |
Proteomic Dynamic Analysis of IBDV-infected Host Cells

| TABLE 1—continued |
|-------------------|
| **Metabolism-associated proteins** |
| **Carbohydrate degradation** |
| **2314 enolase 1** | ENO1 | gi|46048768 | 47.28/47.84 | 6.17/6.09 | 16/15 | 1.25±0.15 | 0.43±0.03** | 0.45±0.06  |
| **2006 triosephosphate isomerase 1** | TPI1 | gi|45382061 | 26.60/26.54 | 6.71/5.25 | 12/16 | 1.10±0.25 | 0.39±0.03* | 0.27±0.03** |
| **5006 6PGL** | 6PGL | gi|71896147 | 26.20/26.77 | 5.56/6.77 | 4/1 | 1.07±0.10 | 0.41±0.03** | 0.33±0.05* |
| **Amino acid transport and metabolism** |
| **4706 thimet oligopeptidase** | TOPA | gi|50798984 | 48.00/83.50 | 8.03/6.51 | 14/11 | 1.00±0.03 | 0.50±0.13* | 0.27±0.03* |
| **0005 glutathione transferases CL2** | GSTCL | gi|46048768 | 25.88/25.67 | 6.85/5.26 | 3/9 | 1.06±0.01 | 0.33±0.04* | 0.28±0.12** |
| **1010 class mu glutathione transferase** | GSTM | gi|7582395 | 25.62/26.23 | 6.9/5.74 | 7/12 | 1.07±0.09 | 0.42±0.05* | 0.36±0.01* |
| **6214 dimethylarginine dimethylaminohydrolase I** | DDAH1 | gi|45383392 | 31.22/37.23 | 5.44/7.11 | 4/7 | 1.28±0.09 | 0.23±0.02* | 0.18±0.02* |
| **4314 B-creatine kinase** | CKB | gi|211235 | 42.24/43.6 | 5.78/6.61 | 5/12 | 1.25±0.25 | 0.36±0.11* | 0.34±0.02** |
| **Lipoprotein metabolism** |
| **7001 apolipoprotein A-I, apoA-I** | APOA-1 | gi|245563 | 28.41/25.70 | 5.57/7.25 | 14/7 | 1.03±0.02 | 0.71±0.87* | 9.44±0.05** |
| **Signal transduction** |
| | | | | | | | | | | | |
lysates of IBDV-infected and mock-infected CEFs at 12, 24, 48, 72, and 96 h p.i. were examined by Western blot analysis with specific antibodies to ubiquitin, Rho-GDI, vimentin, and β-tubulin. In Western blot analysis, CEF polyubiquitin, β-tubulin, and Rho-GDI were detected with their respective mAbs (Fig. 5), whereas vimentin was not detected with the mAb to vimentin (data not shown). The expression of polyubiquitin was induced in IBDV-infected CEFs at 12 h p.i., elevated at 24 h, and reached a peak at 48 h p.i. In contrast, the expression of Rho-GDI was slightly reduced at 12 and 24 h p.i. and significantly inhibited between 48 and 96 h p.i. β-Tubulin (β5 isoform 4) expression was also shown to be up-regulated in the IBDV-infected CEFs. These data agreed with the expression changes shown by the 2-DE analysis.

Immunoctytochemical Validation—To visualize the differentially expressed proteins in IBDV-infected cells, an indirect immunofluorescence assay (IFA) was performed using the mAbs to vimentin, polyubiquitin, β-tubulin, and Rho-GDI. In this analysis, the mAb to vimentin could react with the vimentin of Vero cells but not with that of CEFs, and mAb to β-tubulin could recognize β-tubulin in both CEFs and Vero cells. However, ubiquitin and Rho-GDI in infected or uninfected CEFs and Vero cells were not recognized by the mAbs to ubiquitin and Rho-GDI (data not shown). Thus, we opted to examine the subcellular location of vimentin filaments in IBDV-infected Vero cells and tubulin in IBDV-infected CEFs and Vero cells using double staining IFA. As shown in Fig. 6, the filamentous vimentin was considerably broken down in IBDV-infected Vero cells. Similarly the radial array of microtubules was found to partially or totally disappear in IBDV-infected CEFs and Vero cells. These data demonstrate that the structure of filamentous vimentin and β-tubulin microtubule networks were disassembled in cytopathic IBDV-infected cells.

**DISCUSSION**

Increasing evidence emphasizes comparative proteomics to screen the differentially expressed proteins associated with host cellular pathophysiological processes of virus infection (15). From the literature, it appears that very few studies have been performed to analyze the interplay between IBDV and host cells using proteomics analysis. In our study, we obtained a dynamic overview of the altered protein expression of host cells responding to IBDV infection. The identified cellular proteins function in translational regulation, UPP, cytoskeleton organization, signal transduction, stress response, and macromolecular biosynthesis and as metabolic enzymes (Table I). Notably the data obtained in this study indicate that...
IBDV infection triggers the expression of the cellular UBI and ACT5 genes and turns off the expression of CAPZA1, MAPRE1, AHA1, and UCHL5 genes (Table I and Fig. 2). This is the first report of proteomics analysis performed to study the cellular response to IBDV infection.

**Proteomic Dynamic Analysis of IBDV-infected Host Cells**

**Fig. 2.** Dynamic 2-DE profiles of the differentially expressed proteins in the IBDV-infected cells. Circles indicate the differentially expressed protein spots. C+ and C− indicate the IBDV-infected and uninfected CEFs, respectively.

**Fig. 3.** Classification of the differentially expressed proteins in IBDV-infected CEFs according to their subcellular location and biological function. A and C, functional classification of the differentially expressed proteins in IBDV-infected CEFs. B and D, subcellular location of the differentially expressed proteins in IBDV-infected CEFs.

IBDV infection triggers the expression of the cellular UBI and ACT5 genes and turns off the expression of CAPZA1, MAPRE1, AHA1, and UCHL5 genes (Table I and Fig. 2). This is the first report of proteomics analysis performed to study the cellular response to IBDV infection.

**IBDV Infection Hijacking of the Host Translation Appara...**
EIF2S1 is a eukaryotic translation initiation factor that participates in cap-dependent translation by binding the initiator tRNA to the 40 S ribosomal subunit (31) and plays a central role in the maintenance of a rate-limiting step in cellular mRNA translation (32). EIF4A2 is a eukaryotic translation initiation factor whose function is to melt the 5'-terminal secondary structure of eukaryotic mRNAs to facilitate the attachment of the 40 S ribosomal subunit and to play an essential role in the initiation of the translation of both capped and uncapped mRNA (33, 34). Histidyl-tRNA synthetase (HARS) belongs to the class IIa aminoacyl-tRNA synthetases that are thought to function in cellular mRNA translation by binding ATP (35). This study identified five translation-related proteins in IBDV-infected cells involved in the up-regulation of ARPP0, RPSA, and EIF4A2 and the down-regulation of EIF2S1 and HARS. The reported data showed that the host translational...

Fig. 4. Transcript alteration of the differentially expressed proteins in IBDV-infected CEFs. Total cellular RNA of CEFs with or without IBDV infection was measured by real time RT-PCR analysis. Samples were normalized with histone H5 gene as the control housekeeping gene and with uninfected CEFs at each respective time point as the reference sample. Error bar is standard deviation. Gene symbols indicating different genes refer to Table I.

Fig. 5. Western blot confirmation of representative proteins in IBDV-infected CEFs. A, the expression changes of Rho-GDI, polyubiquitin, and TUBB5 in 2-DE gels. B, the immunoblots of Rho-GDI, polyubiquitin (UBI) and β-tubulin (TUBB) with the mAbs to Rho-GDI, ubiquitin, and β-tubulin, respectively. C, the averaged densitometric intensity of three replicate immunoblots. PM represents prestained protein molecular weight markers. C+ and C− represent the IBDV-infected and uninfected CEFs, respectively. Error bar reveals standard deviation.

tus—EIF2S1 is a eukaryotic translation initiation factor that participates in cap-dependent translation by binding the initiator tRNA to the 40 S ribosomal subunit (31) and plays a central role in the maintenance of a rate-limiting step in cellular mRNA translation (32). EIF4A2 is a eukaryotic translation initiation factor whose function is to melt the 5'-terminal secondary structure of eukaryotic mRNAs to facilitate the attachment of the 40 S ribosomal subunit and to play an essential role in the initiation of the translation of both capped and uncapped mRNA (33, 34). Histidyl-tRNA synthetase (HARS) belongs to the class IIa aminoacyl-tRNA synthetases that are thought to function in cellular mRNA translation by binding ATP (35). This study identified five translation-related proteins in IBDV-infected cells involved in the up-regulation of ARPP0, RPSA, and EIF4A2 and the down-regulation of EIF2S1 and HARS. The reported data showed that the host translational...
machinery was turned off by down-regulating EIF4A2 in cells infected with influenza virus type A, poliovirus, herpes simplex virus, and entero virus 71 (36–38). Interestingly EIF2S1 and HARS were considerably down-regulated in IBDV-infected cells. Hence we infer that IBDV turns off the host translational machinery for initiating its viral translation in infected cells by down-regulating EIF2S1 but not EIF4A2. This hypothesis is supported by evidence that shows that the IBDV VP1 protein interacts with the carboxyl-terminal domain of EIF4A2 (40) and by data that demonstrate that the families of RNA viruses hijack the host translation apparatus to successfully complete viral mRNAs translation (41). Moreover RPSA and ARPP0 up-regulation was also found in cells infected with rice yellow mottle virus (17), FHV (23), and SARS-CoV (25), suggesting that the two proteins may not be specific to IBDV infection-associated cells and tissues.

Alteration of Cytoskeleton Networks—In this study, in the identified microfilament-associated and microtubule-associated proteins, many actin and tubulin proteins were up-regulated, whereas three microfilament-associated proteins (CAPZA1, CNN3, and ACTL6A) were down-regulated, and MAPRE1 was inhibited (Table I). Unlike microfilament- and microtubule-associated cytoskeleton, the class III IF protein vimentin and a nuclear member of IF, namely chicken lamin B2, were greatly decreased. The changes in α-actin (up-regulated ACTA2 and down-regulated alpha actin of cardiac muscle) or TUBA2 or LAMB1 have been detected in enterovirus 71-, SARS-CoV-, and influenza virus-infected cells, respectively (20, 25, 42). Although these proteins may not be specific to IBDV, most of the cytoskeleton alterations detected in IBDV-infected cells were caused by IBDV infection. IFA clearly demonstrated that the vimentin and β-tubulin networks collapse and disperse in IBDV-infected cells (Fig. 6). Hence we speculate that cytoskeletal disruption may be a critical mechanism of IBDV particle release from infected cells. Recent evidence demonstrates that various viruses manipulate and utilize the host cytoskeleton to promote viral infection (43). Several studies have shown that human immunodeficiency virus type 1 protease cleaves the IF vimentin and induces the collapse of vimentin in infected cells (44, 45). Further elucidation is required to determine whether IBDV protease VP4 uses an human immunodeficiency virus-like strategy to cleave vimentin, resulting in highly decreased expression and the collapse of the vimentin network. In addition, it is unclear why ACT5, a third cytoplasmic isoform of the chicken non-muscle actin (46), was induced in the late stages of IBDV infection.

Apolipoprotein A-I (APOA-I), Rho-GDI, and Actin Cytoskeleton—In this study, APOA-I expression was up-regulated in IBDV-infected CEFs. In contrast, the signal transduction proteins Rho-GDI and D4-GDI were considerably down-regulated (Figs. 2 and 5). APOA-I, a major constituent of high density lipoproteins, alters plasma membrane morphology by participating in the reverse transport of cholesterol binding with ATP-binding cassette transporter A1 (47) and activates the small GTP-binding protein Cdc42-associated signaling including APOA-I-induced cholesterol efflux, protein kinases, and actin polymerization (48). Cumulative evidence also shows that the GTPases of the Rho family are key regulatory molecules of the actin cytoskeleton (49) and that Cdc42-activated GTPase induces the collapse of the vimentin network (50). Rho-GDI and D4-GDI were also reported to be members of the Rho-GTPase regulator family that regulate a wide variety of cellular functions by binding and inhibiting Rho GTPases (51). Thus, the activity of GTPase regulating the cytoskeletal networks may have been interfered with by the high expression of APOA-I and down-regulation of both Rho-GDI and D4-GDI during IBDV infection.

UPP Disorders in IBDV-infected Cells—Ubiquitin was described as a heat shock protein playing an important role both during and after stress in CEFs (52). UPP, a major intracellular protein degradation pathway, has recently been implicated in viral infections, including avoidance of host immune surveillance, viral maturation, viral progeny release, efficient viral replication, and reactivation of virus from latency (53). Previous reports considered that the replication of pea seed- borne mosaic virus induces polyubiquitin and HSP70 expression (54). In this study, nine UPP-linked proteins were identified as differentially expressed cellular proteins following IBDV infection (Table I and Fig. 2): the up-regulated polyubiquitin and the down-regulated ubiquitin-conjugating enzyme (HIP2), proteasome 20 S subunit (PSMA2, PSMA6, and PSMB1), proteasome activator subunit (PSME3), proteasome 26 S subunit, ATPase (PSMD5), and deubiquitinating enzyme (UCHL1 and UCHL5). Notably after IBDV infection, most of the UPP-associated proteins involved in ubiquitination and deubiquitination were down-regulated, and UCHL5 was completely inhibited, whereas polyubiquitin was initiated as a newly induced cellular protein (Fig. 4). These data indicate that IBDV infection results in functional disorders of the UPP system as a “cell cleaner,” although the reason for this is unknown. Schlee et al. (22) reported the down-regulation of UCHL3 in Epstein-Barr virus-infected cells, suggesting that IBDV differs from Epstein-Barr virus in how it disturbs cellular UPP. In addition, Leong and Chow (20) found PSMA2 and UCHL3 down-regulation in enterovirus 71-infected cells, revealing that the two proteins may not be specific to IBDV infection.

IBDV Infection and Stress Response Proteins—In this study, two unique HSPB1 spots representing an ATP-independent chaperone were identified during IBDV infection (Table I and Fig. 2). Up-regulated HSPB1 spots have been found in cells infected with African swine fever virus (19), enterovirus 71 (20), or FHV (23). Interestingly one HSPB1 protein spot presented an increase, whereas another HSPB1 protein spot presented a decrease, demonstrating that the up-regulated HSPB1 may not be specific to IBDV infection and that different isoforms or modifications of HSPB1 may play different roles during IBDV infection. The phosphorylated HSPB1 was shown to prevent...
F-actin depolymerization by regulating microfilament dynamics and stabilizing the actin cytoskeleton under stress conditions (55) and to prevent caspase-independent apoptosis (56). In addition, another stress response protein, activator of heat shock 90-kDa protein ATPase homolog 1 (AHAl), was down-regulated or inhibited during IBDV infection (Fig. 2). Previous studies have shown that AHAl is a co-chaperone that stimulates Hsp90 ATPase activity and may affect a step in the endoplasmic reticulum-to-Golgí trafficking and that this Hsp90-associated ATP/GTPase may participate in the regulation of complex formation of Hsp90 (57). Consequently the inhibition of AHAl expression in IBDV-infected cells may lead to impairment of intracellular protein trafficking in infected cells.

Inhibition of RNA Processing, Macromolecular Biosynthesis, and Energy Metabolism—In this experiment, we also found decreased expression of cellular proteins associated with RNA processing and biosynthesis (Table I and Fig. 2), including HNRPNL-binding RNA activity (58), SF3A3 participation in the nuclear mRNA splicing via spliceosome (59), FPS involvement in isoprenoid biosynthesis, participation of SNX6 containing the phox domain in targeting proteins to membranes (60), and MTA. Notably the proteomics data further revealed that the key glycolytic enzymes, including ENO1, ALDOC, AKR, LDHB, and 6PGL, and the key regulatory enzymes involved in amino acid transport and metabolism, including GSTCL2 and CKB, were extensively down-regulated. Based on the information from these proteomics data, we speculate that IBDV replications may extensively inhibit the host cellular metabolic pathways involved in glycolysis and amino acid transport as well as mRNA processing, although ENO1 down-regulation was found in respiratory syncytial virus-infected cells (21).

Conclusion—This study adopted a gel-based proteomics approach to probe the serially changed proteins in IBDV-infected CEFs. It is noteworthy that the comparative proteomics approach allowed for the initial identification of 51 altered cellular proteins during IBDV infection and showed that most of the altered cellular proteins appear to have roles in revealing the viral pathogenesis. Clearly further large scale studies are necessary to understand the roles of the differentially expressed cellular proteins in IBDV infection.

Acknowledgments—We thank Xin-Wen Zhou (Fudan University, Shanghai, China) for help with MALDI-TOF/TOF mass spectrometry and Han-Min Chen for technical help on laser confocal microscopy.

* This work was supported by National Key Basic Research Program of China (Grant 2005CB523203), National Key Technology Research and Development Program of China (Grant 2006BAD06A04), and National Natural Science Foundation of China (Grant No. 30625030). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** The on-line version of this article (available at http://www.mcponline.org) contains supplemental material.

To whom correspondence should be addressed: Inst. of Preventive Veterinary Medicine, Zhejiang University, 268 Kaixuan Rd., Hangzhou 310029, China. Tel.: 86-571-8697-1698; Fax: 86-571-8697-1821; E-mail: jyzhou@zju.edu.cn.

REFERENCES

1. Sall, Y. M. (1991) Immunosuppression induced by infectious bursal disease virus. Vet. Immunol. Immunopathol. 30, 45–50
2. Vasconcelos, A. C., and Lam, K. M. (1994) Apoptosis induced by infectious bursal disease virus. J. Gen. Virol. 75, 1803–1806
3. Tanimura, N., and Sharma, J. M. (1998) In-situ apoptosis in chickens infected with infectious bursal disease virus. J. Comp. Pathol. 118, 15–27
4. Vasconcelos, A. C., and Lam, K. M. (1995) Apoptosis in chicken embryos induced by the infectious bursal disease virus. J. Comp. Pathol. 112, 327–338
5. Jungmann, A., Nieper, H., and Muller, H. (2001) Apoptosis is induced by infectious bursal disease virus replication in productively infected cells as well as in antigen-negative cells in their vicinity. J. Gen. Virol. 82, 1107–1115
6. Nick, H., Cursiefen, D., and Becht, H. (1976) Structural and growth characteristics of infectious bursal disease virus. J. Virol. 18, 227–234
7. Tham, K. M., and Moon, C. D. (1996) Apoptosis in cell cultures induced by infectious bursal disease virus following in vitro infection. Avian Dis. 40, 109–113
8. Fernandez-Arias, A., Martinez, S., and Rodriguez, J. F. (1997) The major antigenic protein of infectious bursal disease virus, VP2, is an apoptotic inducer. J. Virol. 71, 8014–8018
9. Liu, M., and Vakharia, V. N. (2006) Nonstructural protein of infectious bursal disease virus inhibits apoptosis at the early stage of virus infection. J. Virol. 80, 3369–3377
10. Lombardo, E., Maraver, A., Espinosa, I., Fernandez-Arias, A., and Rodriguez, J. F. (2000) VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. Virology 277, 345–357
11. Wong, R. T., Hon, C. C., Zeng, F., and Leung, F. C. (2007) Screening of differentially expressed transcripts in infectious bursal disease virus-induced apoptotic chicken embryonic fibroblasts by using cDNA microarrays. J. Gen. Virol. 88, 1785–1796
12. Gygi, S. P., Rochon, Y., Franza, B. R., and Aebersold, R. (1999) Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19, 1720–1730
13. Shackelford, J., and Pagano, J. S. (2005) Targeting of host-cell ubiquitin pathways by viruses. Essays Biochem. 41, 139–156
14. Meredith, D. M., Lindsay, J. A., Halliburton, I. W., and Whitaker, G. R. (1991) Post-translational modification of the tegument proteins (VP13 and VP14) of herpes simplex virus type 1 by glycosylation and phosphorylation. J. Gen. Virol. 72, 2771–2775
15. Maxwell, K. L., and Frappier, L. (2007) Viral proteomics. Microb. Mol. Biol. Rev. 71, 398–411
16. Tang, H., Peng, T., and Wong-Staal, F. (2002) Novel technologies for studying virus-host interaction and discovering new drug targets for HCV and HIV. Curr. Opin. Pharmacol. 2, 541–547
17. Ventelon-Debout, M., Delalande, F., Brizard, J. P., Diemer, H., Van Dorselaer, A., and Brugidou, C. (2004) Proteome analysis of cultivar-specific deregulations of Oryza sativa indica and O. sativa japonica cellular suspensions undergoing rice yellow mottle virus infection. Proteomics 4, 216–225
18. Casado-Vela, J., Selles, S., and Martinez, R. B. (2006) Proteomic analysis of tobacco mosaic virus-infected tomato (Lycopersicon esculentum M.) fruits and detection of viral coat protein. Proteomics 6, Suppl. 1, S196–S206
19. Alfonso, P., Rivera, J., Hernaez, B., Alonso, C., and Escrivano, J. M. (2004) Identification of cellular proteins modified in response to African swine fever virus infection by proteomics. Proteomics 4, 2037–2048
20. Leong, W. F., and Chow, V. T. (2006) Transcriptomic and proteomic analyses of rhabdomyosarcoma cells reveal differential cellular gene expression in response to enterovirus 71 infection. Cell. Microbiol. 8, 565–580
21. Brasier, A. R., Spratt, H., Wu, Z., Boldogh, I., Zhang, Y., Garofalo, R. P., Casola, A., Pashmi, J., Haag, A., Luxon, B., and Kurosky, A. (2004) Nuclear heat shock response and novel nuclear domain 10 reorganization in respiratory syncytial virus-infected a549 cells identified by high-
37. Garfinkel, M. S., and Katze, M. G. (1992) Translational control by influenza virus. Selective and cap-dependent translation of viral mRNAs in infected cells. 

38. Griffin, T. J., Gygi, S. P., Ideker, T., Rist, B., Eng, J., Hood, L., and Mann, M. (2003) Global mass spectrometric analysis of yeast translation products. 

39. Kuyumcu-Martinez, N. M., Van Eden, M. E., Younan, P., and Lloyd, R. E. (2004) Cleavage of poly(A)-binding protein by poliovirus 3C protease inhibits host cell translation: a novel mechanism for host translation shutoff. Mol. Cell. Biol. 24, 1779–1790

40. Tacken, M. G., Thomas, A. A., Peeters, B. P., Rottier, P. J., and Boot, H. J. (2004) VP1, the RNA-dependent RNA polymerase and genome-linked protein of infectious bursal disease virus, interacts with the carboxy-terminal domain of translational eukaryotic initiation factor 4AII. Arch. Virol. 149, 2245–2260

41. Bushell, M., and Samow, R. P. (2002) Hijacking the translation apparatus by RNA viruses. J. Cell Biol. 158, 395–399

42. Baas, T., Baskin, C. R., Diamond, D. L., Garcia-Sastre, A., Bielefeldt-Ohmann, H., Tumpey, T. M., Thomas, M. J., Carter, V. S., Teal, T. H., Van Hoeven, N., Proll, S., Jacobs, J. M., Caldwell, Z. R., Gritsenko, M. A., Hukkainen, R. R., Camp, D. G., II, Smith, R. D., and Katze, M. G. (2006) Integrated molecular signature of disease: analysis of influenza virus-infected macrophages through functional genomics and proteomics. J. Virol. 80, 10813–10828

43. Radtke, K., Dohner, K., and Sodeik, B. (2006) Viral interactions with the cytoskeleton: a hitchhiker’s guide to the cell. Cell. Microbiol. 8, 387–400

44. Shoemaker, R. L., Honer, B., Stoller, T. J., Kesselmeier, C., Miedel, M. C., Traub, P., and Graves, M. C. (1990) Human immunodeficiency virus type 1 protease cleaves the intermediate filament proteins vimentin, desmin, and glial fibrillary acidic protein. Proc. Natl. Acad. Sci. U. S. A. 87, 3666–3670

45. Honer, B., Shoemaker, R. L., and Traub, P. (1991) Human immunodeficiency virus type 1 protease microinjected into cultured human skin fibroblasts cleaves vimentin and affects cytoskeletal and nuclear architecture. J. Cell Sci. 100, 799–807

46. Bergsma, D. J., Chang, K. S., and Schwartz, R. J. (1985) Novel chicken actin gene: third cytoplasmic isoform. Mol. Cell. Biol. 5, 1151–1162

47. Wang, N., Silver, D. L., Costeloe, K., and Tall, A. R. (2000) Specific binding of Apoα-1, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J. Biol. Chem. 275, 33053–33058

48. Nofer, J. R., Remaley, A. T., Feuerborn, R., Wolinnska, I., Engel, T., von Eckardstein, A., and Assmann, G. (2006) Apolipoprotein A-I activates Cdc42 signaling through the ABCA1 transporter. J. Lipid Res. 47, 794–803

49. Hall, A. (1998) Rho GTPases and the actin cytoskeleton. Science 279, 509–514

50. Meriane, M., Mary, S., Comunale, F., Vignal, E., Fort, P., and Gauthier-Rouviere, C. (2000) Cdc42Hs and Rac1 GTPases induce the collapse of the vimentin intermediate filament network. J. Biol. Chem. 275, 33046–33052

51. Van Aelst, L., and D’Souza-Schorey, C. (1997) Rho GTPases and signaling networks. Genes Dev. 11, 2295–2322

52. Bond, U., Agell, N., Haas, A. L., Redman, K., and Schlesinger, M. J. (1988) The ubiquitin-proteasome pathway in viral infections. Proc. Natl. Acad. Sci. U. S. A. 85, 15289–15293

53. Größl, A., Lambart, H., Gingras-Breton, G., Lavoie, J. N., Huot, J., and Landry, J. (1997) Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. J. Cell Sci. 110, 357–368

54. Mossner, D. S., and Morimoto, R. I. (2004) Molecular chaperones and the stress of oncogenesis. Oncogene 23, 2907–2918

55. Panaretou, B., Siligardi, G., Meier, P., Maloney, A., Sullivan, J. K., Singh, S., Millson, S. H., Clarke, P. A., Naba-Hansen, S., Stein, R., Cramer, R., Mollapour, M., Workman, P., Piper, P. W., Pearl, L. H., and Prodromou, C. (2002) Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. Mol. Cell. 10, 1307–1318

56. Dreyfuss, G., Matunis, M. J., Pinol-Roma, S., and Burd, C. G. (1993) hnRNP proteins and the biogenesis of mRNA. Annu. Rev. Biochem. 62, 289–321

57. Yuan, X., Kuramitsu, Y., Furumoto, H., Zhang, X., Hayashi, E., Fujimoto, M., and Nakamura, K. (2007) Nuclear protein profiling of Jurkat cells during heat stress-induced apoptosis by 2-DE and MS/MS. Electrophoresis 28, 2018–2026