An interactome map of the nucleocapsid protein from a highly pathogenic North American porcine reproductive and respiratory syndrome virus strain generated using SILAC-based quantitative proteomics

Stefanie S. Jourdan, Fernando Osorio and Julian A. Hiscox

Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK
School of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA

Positive strand RNA viruses replicate in the cytoplasm of an infected cell and encode nucleocapsid proteins. These proteins function to promote encapsidation of the RNA genome and virus particle assembly as well as playing potential roles in viral RNA synthesis. Nucleocapsid proteins can also associate with cellular proteins and signaling cascades. The arterivirus nucleocapsid (N) protein is no exception and localizes to both the cytoplasm and the nucleus in virus-infected cells. This study generated an interactome map of the N protein from a highly virulent North American strain of porcine reproductive and respiratory syndrome virus (PRRSV). This is a major pathogen of swine resulting in significant morbidity and mortality. Crucial to the study was the use of SILAC coupled to affinity purification using GFP-traps and LC-MS/MS. This approach has not been applied before to the investigation of host/viral protein interactomes and this study revealed that the PRRSV N protein interacts with the host cell protein synthesis machinery especially at the level of translation initiation as well as with the RNA post-transcriptional modification machinery. Applications of the dataset can include studies of virus/host interactions and the design of live attenuated recombinant vaccines.

Keywords:
Bioinformatics / MASCOT / Microbiology / Multiprotein complex / Stable isotope labelling

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in pregnant sows, a high mortality in piglets and respiratory disease in pigs of any age [1]. The spread of PRRSV can theoretically be controlled by vaccination. Although inactivated vaccines have been used to attempt to contain the disease, live attenuated vaccines are the only type capable of establishing protective immunity [2]. However, currently available live vaccine strains are unstable and sometimes revert to virulent phenotypes in vaccinated animals. Emerging research suggests that live vaccines based on recombinant viruses with selected multiple attenuating mutations offer the best potential for future vaccine efforts. Due to the high losses in production resulting from PRRSV infection, the virus is of great economic importance. PRRSV belongs to the family of arteriviruses that are grouped together with the Corona- and Toroviruses into the order Nidovirales. During arterivirus (and coronavirus) infection, one of the most abundant viral proteins within the cell is the N protein that plays essential roles in the virus life cycle including encapsidation of the viral RNA [3]. Although these viruses replicate in the cytoplasm, the N protein has been...
observed to localize to the nucleolus in a wide range of corona and arteriviruses [4–8].

Nidovirus proteins have also been reported to interact with cellular proteins and signaling cascades. For PRRSV predominant amongst these is the potential involvement of N protein in modulation of host cell function and recruiting cellular factors to facilitate virus replication [9]. Several amino acid motifs on the N protein have been identified that are involved in the cytoplasmic/nuclear/nucleolar trafficking of the protein and also interactions with cellular proteins [10–12]. N protein has been shown to interact with importin-α and importin-β [12], the nucleolar protein fibrillarin, [13] and several others [9].

Given the importance of the N protein in the life cycles of arteriviruses and the role of these viruses in health and food security, we decided to generate a cellular interactome map of the arterivirus N protein using enhanced green fluorescent protein (EGFP)-trap technology coupled to SILAC to help distinguish background binding from potentially specific interactions [14]. LC-MS/MS was used to identify and quantify proteins, and binding to selected cellular proteins was validated using Western blot in separate technical non-labeled biological replicates. The potential use of the dataset was then demonstrated. The EGFP-N protein fusion approach was taken in order to fully utilize the high affinity of the GFP-trap (Chromotek), which consists of a single domain anti-GFP antibody conjugated to an agarose bead matrix. This approach was also taken over selectively immuno-precipitating N protein from PRRSV-infected cells as we wanted to examine the specific interaction of N protein with cellular components. In PRRSV infected cells, N protein may interact with other viral proteins which themselves will bind to cellular proteins.

In order to generate an N protein fused to EGFP, the PRRSV N gene was cloned upstream of the recombinant GFP (RGF) gene from the genome of a pathogenic PRRSV template produced by a cDNA infectious clone derived from isolate NVSL #97-7895 [15].Alanine substitution mutants of N protein to illustrate applications of the dataset were generated using Western blot in separate technical non-labeled biological replicates. The potential use of the dataset was then demonstrated. The EGFP-N protein fusion approach was taken in order to fully utilize the high affinity of the GFP-trap (Chromotek), which consists of a single domain anti-GFP antibody conjugated to an agarose bead matrix. This approach was also taken over selectively immuno-precipitating N protein from PRRSV-infected cells as we wanted to examine the specific interaction of N protein with cellular components. In PRRSV infected cells, N protein may interact with other viral proteins which themselves will bind to cellular proteins.

N-EGFP fusion protein localized to the nucleolus and the cytoplasm in 293-T cells (Supporting Information Fig. 1) was identical to the pattern found for this protein in Marc145 cells [6,13] and other cell types, such as Vero cells [18]. GFP-Trap beads were used to carry out pull-down experiments using cell lysates from cells expressing N-EGFP or EGFP. Quantitative proteomic analysis using LC-MS/MS of the cellular proteins was eluted off the beads identified 224 cellular proteins (Supporting Information Table S1). Confidence in protein identification was considered high if the PEP (posterior error probability) score was close to zero. In several cases very similar proteins belonging to the same family, e.g. PABP1 and PABPC4 may be assigned to a similar set of peptides and this information is presented in Supporting Information Table S1 (columns A and B). However, use of biological replicates and specific antibodies can help distinguish between these proteins (see below). Raw data was deposited with the PRoteomics IDentiﬁcations database (PRIDE) [19] using the PRIDE converter [20]. This dataset was also deposited with the IMEx molecular interaction database through IntAct [21,22] and assigned the identifier IM-16317.

Fifty-six cellular proteins were identified and quantified by two or more peptides and were enriched two-fold or more in the N-EGFP fraction compared to the EGFP fraction. These proteins were grouped into categories with similar functions (Table 1) and used in further analysis to validate the dataset and demonstrate its application. Apart from background binding, a number of these proteins may form dynamic or weak interactions with N protein, and hence not be enriched in the N-EGFP pull-down compared to the EGFP pull-down control (Supporting Information Table S1). Present amongst these are importin-5 (0.76), nucleolin (0.74), and ribosomal proteins that have previously been shown to interact with arterivirus and/or coronavirus N proteins [9] (also see Table 1).

Bioinformatic analysis of the dataset using IPA indicated that N-EGFP associated with proteins involved a number of different molecular and cellular functions including: RNA post-transcriptional modification (splicing) (24 molecules, p-value 1.41 × 10^{-28} – 4.19 × 10^{-7}), protein synthesis (11 molecules, p-value 2.09 × 10^{-8} – 3.51 × 10^{-2}), RNA trafficking (8 molecules, p-value 1.64 × 10^{-7} – 2.12 × 10^{-2}), and gene expression (22 molecules, p-value 7.75 × 10^{-6} – 4.53 × 10^{-2}) (Fig. 1A). Many of these proteins are shared between the different functions and can be linked by known protein–protein interactions (Fig. 1B).

To confirm the LC-MS/MS data, pull-downs were repeated in a separate experiment in the absence of label and were analyzed by Western blot using antibodies specific against a selection of hits representing different functional classes focused on translation and splicing, e.g. poly(A) binding protein (PABP), inducible PABP (iPABP), translation initiation factor 4E (eIF4E), hnRNP A1, and NONO (Fig.2A). These were selected on the basis of interest but also representing
Table 1. Proteins identified in the N-EGFP Trap and differentiated from background EGFP binding using SILAC

| Protein ID | Gene name      | Protein name                                      | N-EGFP / EGFP | Pep. Seq. cov. (%) | PEP    | Notes                                                                 |
|------------|----------------|---------------------------------------------------|---------------|-------------------|--------|----------------------------------------------------------------------|
|            |                | **Translation**                                   |               |                   |        |                                                                      |
| IPI00555747.1 | PABPC4 | Inducible polyadenylate-binding protein 1          | 17            | 30                | 41.5   | Binds to the poly(A) tail                                            |
| IPI00008524.1 | PABPC1 | Polyadenylate-binding protein 1                    | 16            | 34                | 48.1   | Binds to the poly(A) tail. Shown to bind to the 3' untranslated region and polyA tail of coronavirus genomes [33, 34] |
| IPI00646377.1 | EIF4G3 | Eukaryotic translation initiation factor 4 gamma 3 | 8             | 4                 | 2.5    | Involved in the recognition of the mRNA cap                         |
| IPI00873680.2 | EIF4E | Eukaryotic translation initiation factor 4E        | 5             | 3                 | 12.9   | Recognizes the mRNA cap and facilitates ribosome's binding by unwinding mRNA secondary structure            |
| IPI00719752.1 | EIF3B | Eukaryotic translation initiation factor 3 subunit B | 4             | 11                | 14     | Part of the elf-3 complex that facilitates recruitment of mRNA to the 43S pre-initiation complex for AUG recognition |
| IPI00646839.1 | EIF3C | Eukaryotic translation initiation factor 3 subunit C | 5             | 11                | 12.7   | As above                                                              |
| IPI00465233.1 | EIF3EIP | Eukaryotic translation initiation factor 3, subunit E interacting protein | 5 | 3 | 6.6 | 7.4E-16 | As above |
| IPI00871852.1 | EIF4A1 | ATP-dependent RNA helicase eIF4A-1                 | 4             | 7                 | 21.7   | Involved in cap recognition and binding of mRNA to the ribosome. Found in PRRSV virions [32] |
| IPI00412343.2 | FMR1 | Fragile X mental retardation 1 protein             | 2             | 3                 | 4.9    | 0.0003 Repressor of translation, binds to the CAP                   |
| IPI00418313.3 | ILF3 | Interleukin enhancer-binding factor 3              | 2             | 13                | 16.3   | Translation inhibitory protein. Can complex with HNRNPs, nucleolin and other proteins |
|            |                | **mRNA stability**                                |               |                   |        |                                                                      |
| IPI00399170.1 | UPF1 | ATP-dependent helicase RENT1                      | 4             | 20                | 22     | Degradation of mRNAs containing premature stop codons                |
| IPI00784170.1 | DHX36 | DEAH box protein 36                               | 2             | 6                 | 7      | Degradation and deadenylation of mRNAs                               |
| IPI00479786.5 | KHSRP | Far upstream element-binding protein 2             | 2             | 7                 | 11.1   | Involved in mRNA trafficking, degradation of unstable mRNAs         |
| IPI00301936.4 | ELAVL1 | ELAV-like protein 1                               | 5             | 5                 | 16.4   | Involved in mRNA stabilization, specifically to FOS and IL3 mRNAs     |
| IPI00008557.5 | IGF2BP1 | Insulin-like growth factor 2 mRNA-binding protein 1 | 4             | 20                | 40.2   | mRNA trafficking and stability, can function in stress granules     |
| IPI00658000.2 | IGF2BP3 | Insulin-like growth factor 2 mRNA-binding protein 3 | 3             | 12                | 25.9   | Role in mRNA stability binds to the 5' UTR of IGF2 mRNA and the 3' UTR of CD44 mRNA |
| IPI00797384.2 | LARP4 | La-related protein 4                              | 2             | 3                 | 2.8    | 6.1xE-19 Binds with poly(A) RNA and interacts with PABP. Can promote RNA stability |
| IPI00032355.3 | PUM1 | Pumilio homolog 1 (Drosophila)                    | 2             | 2                 | 2.1    | 0.014 Regulates translation and mRNA stability by binding the 3' UTR of mRNA targets |
|            |                | **Heterogeneous nuclear ribonucleoproteins**      |               |                   |        |                                                                      |
| IPI00011274.3 | HNRPD1 | Heterogeneous nuclear ribonucleoprotein D-like     | 3             | 5                 | 11.7   | 1.4E-18 Promotes transcriptional repression, binds to RNA molecules that contain AU-rich elements (AREs) found within the 3 UTRs of many cytokine mRNAs |

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| Protein ID     | Gene name                                | Protein name                                                                 | N-EGFP / EGFP | Pep. Seq. cov.(%) | PEP       | Notes                                                                 |
|---------------|------------------------------------------|-----------------------------------------------------------------------------|---------------|------------------|-----------|------------------------------------------------------------------------|
| IPI00216746.1 | HNRNPK                                   | Heterogeneous nuclear ribonucleoprotein K                                   | 3             | 12               | 35.6      | 1.3E-87                                                              |
|               |                                          |                                                                             |               |                  |           | Major pre-mRNA binding protein and has a high affinity for poly(C) sequences |
| IPI00479191.2 | HNRNPH1                                  | Heterogeneous nuclear ribonucleoprotein H                                   | 3             | 6                | 19.5      | 7.2E-69                                                              |
|               |                                          |                                                                             |               |                  |           | Mediates pre-mRNA alternative splicing regulation                      |
| IPI00013877.2 | HNRNPH3                                  | Heterogeneous nuclear ribonucleoprotein H3                                  | 3             | 2                | 8.4       | 1.2E-09                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in splicing and heat shock-induced splicing arrest            |
| IPI00396378.3 | HNRNPA2B1                                | Heterogeneous nuclear ribonucleoproteins A2/B1                              | 4             | 15               | 45        | 7.0E-74                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in pre-mRNA processing and found in the nucleolus. Interacts with the 3' end of the coronavirus genome [34] |
| IPI00215965.2 | HNRNPA1                                  | Heterogeneous nuclear ribonucleoprotein A1                                  | 3             | 16               | 43        | 9.6E-113                                                             |
|               |                                          |                                                                             |               |                  |           | May modulate splice sites selection and packaging of pre-mRNA into hnRNP particles. Interacts with the 3' end of the coronavirus genome [34] and sites of transcription initiation [35,36]. Binds to SARS-coronavirus N protein [37]. May regulate coronavirus RNA synthesis [38] |
| IPI00419373.1 | HNRNPA3                                  | Heterogeneous nuclear ribonucleoprotein A3                                  | 3             | 6                | 25.7      | 5.4E-34                                                              |
|               |                                          |                                                                             |               |                  |           | Functions in trafficking RNA and pre-mRNA splicing                   |
| IPI00003881.5 | HNRNPF                                   | Heterogeneous nuclear ribonucleoprotein F                                   | 2             | 3                | 10.6      | 4.7E-43                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in pre-mRNA processing and regulation of alternative splicing events |
| IPI00011913.1 | HNRNPA0                                  | Heterogeneous nuclear ribonucleoprotein A0                                  | 2             | 4                | 17.7      | 1.7E-48                                                              |
|               |                                          |                                                                             |               |                  |           | Component of ribonucleosomes                                          |
| IPI00304692.1 | RBMX                                    | Heterogeneous nuclear ribonucleoprotein G                                   | 2             | 2                | 6.6       | 6.9E-07                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in pre-mRNA splicing                                         |
| IPI00477313.3 | HNRNPC                                  | Heterogeneous nuclear ribonucleoproteins C1/C2                              | 2             | 5                | 19.6      | 2.2E-48                                                              |
|               |                                          |                                                                             |               |                  |           | Binds pre-mRNA and nucleates the assembly of 40S hnRNP particles       |
| Splicing      |                                          |                                                                             |               |                  |           |                                                                        |
| IPI00216613.1 | SFPQ                                    | Polypyrimidine tract-binding protein-associated-splicing factor             | 4             | 10               | 17.5      | 1.6E-86                                                              |
|               |                                          |                                                                             |               |                  |           | Also termed SFPQ, interacts with NONO, DNA- and RNA binding protein, involved in several nuclear processes including splicesome formation |
| IPI00183626.8 | PTBP1                                   | Polypyrimidine tract-binding protein 1                                      | 4             | 11               | 26.8      | 4.1E-26                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in pre-mRNA splicing and binds to the polypyrimidine tract of introns. May promote RNA looping. Interacts with the 3' end of the arterivirus genome [39]. Interacts with the 5' end [40] and 3' end of the coronavirus genome, and silencing resulted in a reduction in viral RNA synthesis [34] |
| IPI00215884.4 | SRSF1                                   | Splicing factor, arginine/serine-rich 1                                   | 4             | 6                | 29.8      | 3.5E-18                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in splicing                                                 |
| IPI00010204.1 | SRSF3                                   | Splicing factor, arginine/serine-rich 3                                   | 4             | 3                | 24.4      | 9.8E-11                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in splicing                                                 |
| IPI00000015.2 | SRSF4                                   | Splicing factor, arginine/serine-rich 4                                   | 3             | 2                | 3.2       | 3.8E-06                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in splicing                                                 |
| IPI00003377.1 | SRSF7                                   | Splicing factor, arginine/serine-rich 7                                   | 4             | 2                | 8.8       | 0.0001                                                               |
|               |                                          |                                                                             |               |                  |           | Involved in splicing                                                 |
| IPI00304596.3 | NONO                                    | Non-POU domain-containing octamer-binding protein (NONO)                   | 4             | 12               | 25.3      | 1.7E-57                                                              |
|               |                                          |                                                                             |               |                  |           | Involved in pre-mRNA splicing                                         |
### Table 1. Continued

| Protein ID       | Gene name                                      | Protein name                                                                 | N-EGFP / Pep. Seq. cov. (%) | PEP     | Notes                                                                 |
|------------------|-----------------------------------------------|------------------------------------------------------------------------------|-----------------------------|---------|-----------------------------------------------------------------------|
| IPI00011550.1    | ZCCHC3                                        | Zinc finger CCHC domain-containing protein 3                                 | 2                           | 2       | 6.4                                                                  | 3.8E-05 | May be involved in pre-mRNA splicing                                 |
| IPI00643351.1    | YBX1                                          | Nuclease-sensitive element-binding protein 1                                 | 2                           | 9       | 43                                                                   | 4.7E-115| Can determine splice site selection                                  |
| IPI00294536.2    | STRAP                                         | Serine-threonine kinase receptor-associated protein                          | 2                           | 3       | 12.4                                                                 | 1.6E-10 | Required for pre-mRNA splicing and formation of splicesomal snRNP in the cytoplasm |

**Dead/DeaH RNA helicases**

Characterized by containing the conserved motif Asp-Glu-Ala-Asp (DEAD). Involved in alteration of RNA secondary structure from translation to splicing.

| Protein ID       | Gene name                                      | Protein name                                                                 | N-EGFP / Pep. Seq. cov. (%) | PEP     | Notes                                                                 |
|------------------|-----------------------------------------------|------------------------------------------------------------------------------|-----------------------------|---------|-----------------------------------------------------------------------|
| IPI00293616.3    | DDX3X                                         | DEAD box protein 3                                                           | 3                           | 10      | 18.3                                                                 | 2.3E-120| ATP-dependent RNA helicase                                           |
| IPI00017617.1    | DDX5                                          | DEAD box protein 5                                                           | 3                           | 14      | 23.8                                                                 | 3.2E-73 | ATP-dependent RNA helicase found in the spliceosome C complex        |
| IPI00844578.1    | DHX9                                          | DEAH box protein 9                                                           | 2                           | 32      | 29.8                                                                 | 1.4E-198| Unwinds RNA in a 3’ to 5’ direction. Promotes MYC mRNA stability    |
| IPI00651653.1    | DDX17                                         | DEAD box protein 17                                                          | 4                           | 16      | 22.8                                                                 | 1.1E-72 | ATP-dependent RNA helicase                                           |
| IPI00411733.4    | DHX30                                         | DEAH box protein 30                                                          | 2                           | 13      | 12.8                                                                 | 2.3E-31 | ATP-dependent RNA helicase, identified in a complex with TFAM and SSBP1 |

**RNA binding**

| Protein ID       | Gene name                                      | Protein name                                                                 | N-EGFP / Pep. Seq. cov. (%) | PEP     | Notes                                                                 |
|------------------|-----------------------------------------------|------------------------------------------------------------------------------|-----------------------------|---------|-----------------------------------------------------------------------|
| IPI00260715.5    | FUS                                           | RNA-binding protein FUS                                                      | 3                           | 4       | 10.8                                                                  | 1.3E-19 | binds DNA and RNA                                                    |
| IPI00783271.1    | LRP5                                          | Leucine-rich PPR motif-containing protein, mitochondrial                    | 3                           | 12      | 11                                                                   | 4.7E-58 | binds to HNRPA1-associated poly(A) mRNAs, and also in mitochondria to polyA |
| IPI00185919.3    | LARP5                                         | La-related protein 1                                                         | 12                          | 28      | 31.5                                                                 | 4.8E-132| contains a La motif, involved in RNA binding. Co-localizes with P bodies, which function in RNA degradation |

**Other proteins**

| Protein ID       | Gene name                                      | Protein name                                                                 | N-EGFP / Pep. Seq. cov. (%) | PEP     | Notes                                                                 |
|------------------|-----------------------------------------------|------------------------------------------------------------------------------|-----------------------------|---------|-----------------------------------------------------------------------|
| IPI00444452.3    | MOV10                                         | Putative helicase MOV-1                                                      | 3                           | 3       | 3.6                                                                  | 5.9E-11 | Probable helicase. Part of the RNA-induced silencing complex (RISC)  |
| IPI00641950.3    | GNB2L1                                        | Guanine nucleotide-binding protein subunit beta-2-like 1                     | 4                           | 13      | 50.1                                                                 | 3.2E-97 | Anchors activated PKC to the cytoskeleton, acts as a platform for SRC activation or inactivation |
| IPI00789551.1    | MATR3                                         | Matrin-3                                                                     | 3                           | 6       | 9.7                                                                  | 1.1E-23 | Associates with NONO and involved in the nuclear retention of defective RNAs |
| IPI00083708.3    | PRRC2C                                        | BAT2 domain-containing protein 1                                             | 3                           | 2       | 0.8                                                                  | 4.9E-07 | May function in the regulation of gene expression                    |
| IPI00216689.2    | PCB2                                          | Poly (rC)-binding protein 2                                                  | 2                           | 6       | 23.2                                                                 | 1.6E-23 | Negatively regulates cellular antiviral responses mediated by MAVS signaling |
| IPI00005198.2    | ILF2                                          | Interleukin enhancer-binding factor 2                                       | 2                           | 3       | 10.3                                                                 | 1.5E-10 | Transcription factor                                                 |
| IPI00879750.1    | SNRPD3                                        | Small nuclear ribonucleoprotein Snm D3                                       | 2                           | 3       | 22.9                                                                 | 2.2E-15 | Part of the U7 snRNP complex, identified in the spliceosome C complex |
| IPI00465363.1    | ATXN2L                                        | Ataxin-2-like protein                                                        | 2                           | 3       | 2.8                                                                  | 4.1E-12 | Unknown function                                                     |

Only proteins showing a binding ratio greater than two or more and identified by two or more peptides are shown. Detailed are the protein ID, protein name, binding ratio, number of individual peptides used to identify the protein (pep.), the percentage sequence coverage on the protein this represents (Seq. Cov. [%]), the posterior error probability (PEP) that is used to calculate the false discovery rate and brief notes on the protein function (generally taken from Uniprot). For interpretation, proteins are grouped into functional categories and/or classes. Notes refer to actual/potential protein function and also indicate where an interaction has been shown to occur previously with PRRSV, arteriviruses, or related coronaviruses.
proteins identified from a large and small number of peptides and ratios, e.g. iPABP (30 peptides and 17-fold enhanced) and eIF4E (3 peptides and 5-fold enhanced). They indicated that N-EGFP interacted with protein components of the translation initiation complex (Fig. 2B). The interaction of N protein with the selected cellular proteins was also assessed by disulfide reduction in the pull down reactions. N protein has been reported to multimerize, particularly into dimers [23] with one mechanism through the role of potential disulfide linkages [24,25]. Therefore disruption of disulfide bridge formation would give an indication of whether binding to the cellular protein required multimeric N protein. No difference between the presence and absence of DTT was observed with any of the selected protein, apart from eIF4E, which appeared to bind less well to the N protein in the presence of DTT (Fig. 2A).

Given the role of the N protein in cytoplasmic/nuclear/nucleolar trafficking and previous studies showing the importance of some of these identified cellular proteins (that are found in the nucleus) in the related coronavirus biology (Table 1), it is tempting to speculate that the N protein maybe recruiting nuclear proteins to facilitate virus biology. Knowledge of N/cellular protein interactions can also be used to generate live recombinant vaccines based on attenuating mutations. The use of reverse genetics in PRRSV [15, 26] and the introduction of attenuating mutations [27, 28], including the N gene [10, 11], is a clearly established strategy for investigating PRRSV
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Figure 2. Validation of MS data. (A) Western blots were carried out on the bound fraction from cell lysates containing either EGFP or N-EGFP. Lysates were prepared, or with the addition or absence of DTT to a final concentration of 2 mM (+DTT). In the case of pull-downs carried out under reducing conditions (+DTT) also the dilution and wash buffer contained 2 mM DTT. (B) Schematic of the cap-dependent translation initiation complex depicting an RNA (black line) with its poly(A) tail and its 7-methylguanosine cap (7mG). Shown in complex with the RNA are the PABP that binds to the mRNA’s poly(A) tail as well as to the initiation factor 4G. As eIF4G binds to eIF4E which interacts with the 7mG, circularization of the mRNA is achieved. eIF3 and 4A interact with eIF4G allowing for the recruitment of the 40S ribosomal subunit.

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