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# Resolution of the cellular proteome of the nucleocapsid protein from a highly pathogenic isolate of porcine reproductive and respiratory syndrome virus identifies PARP-1 as a cellular target whose interaction is critical for virus biology

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major threat to the swine industry and food security worldwide. The nucleocapsid (N) protein is a major structural protein of PRRSV. The primary function of this protein is to encapsidate the viral RNA genome, and it is also thought to participate in the modulation of host cell biology and recruitment of cellular factors to facilitate virus infection. In order to the better understand these latter roles the cellular interactome of PRRSV N protein was defined using label free quantitative proteomics. This identified several cellular factors that could interact with the N protein including poly [ADP-ribose] polymerase 1 (PARP-1), a cellular protein, which can add adenosine diphosphate ribose to a protein. Use of the PARP-1 small molecule inhibitor, 3-AB, in PRRSV infected cells demonstrated that PARP-1 was required and acted as an enhancer factor for virus biology. Serial growth of PRRSV in different concentrations of 3-AB did not yield viruses that were able to grow with wild type kinetics, suggesting that by targeting a cellular protein crucial for virus biology, resistant phenotypes did not emerge. This study provides further evidence that cellular proteins, which are critical for virus biology, can also be targeted to ablate virus growth and provide a high barrier for the emergence of drug resistance.



## 1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a global threat to the swine industry and vaccination provides limited protection. During PRRSV infection one of the most abundant viral proteins produced within the cell is the nucleocapsid (N) protein. This protein

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has important roles in the virus life cycle and may have critical interactions with the host cell (Rowland et al., 1999; Sun et al., 2012). At a basic level N protein complexes with virus genomic RNA to form the ribonucleocapsid core and as such is an integral component of the enveloped virus particle (Song et al., 2011). Because the N protein forms a close association with genomic RNA and other virus proteins such as those involved in replication and components of the viral envelope, the protein has been postulate to play an important role in virus RNA synthesis and virion assembly, perhaps in the modulation of these events (Lee et al., 2006).

The interaction of N protein and the host cell has been supported by several studies showing the association of this viral protein with cellular proteins including those involved in nuclear import such as importin-α and importin-β, the nucleolar protein fibrillarin (Yoo et al., 2003), and inhibitor of MyoD (a protein belonging to myogenic regulatory factors) family-a domain-containing protein (Song et al., 2009). Interactome analysis of the N protein using over expression and EGFP-trapping coupled to LC–MS/MS revealed further potential interactions including association with proteins involved in translation initiation and RNA post-transcriptional modification, including poly(A) binding protein (PABP), inducible PABP (iPABP), translation initiation factor 4E (eIF4E) and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) (Jourdan et al., 2012a). Use of RNA silencing of PABP mRNA and ablation of the protein in PRRSV infected cells highlighted a positive role for this protein in infection and demonstrated that host cell proteins are critical for virus biology (Wang et al., 2012).

In order to generate a more comprehensive analysis of cellular proteins that could complex with PRRSV N protein we used an interactome approach in which N protein was expressed and purified exogenously and used as bait. Whilst over-expression analysis coupled to EGFP traps can provide a useful way of determining the interactome of a particular viral (or cellular) protein (Jourdan et al., 2012a; Wu et al., 2012), over-expression can turn on cellular stress pathways and this may lead to a potential bias in the interactome. To identify potential cellular protein interactions with PRRSV N protein, recombinant expressed and purified protein was therefore used as bait and bound to nickel affinity beads and incubated with HEK293T cellular lysate. Label free proteomics was used to both identify and quantify proteins that associated with the N protein bait and the UBC9 control bait. One hundred and eight proteins were identified by two or more peptides bound to the N protein bait which were also four fold or more abundant than binding with the UBC9 control bait. Eighteen proteins were found to be common between this analysis and a previous analysis using EGFP-N expressed in cells as bait (Jourdan et al., 2012a), including PABPC4 and PABPC1 as the top hits.

One of the unique proteins identified in the current study was Poly [ADP-ribose] polymerase 1 (PARP-1), which although normally associated with DNA virus (e.g. (Grady et al., 2012; Ohsaki et al., 2004; Tempera et al., 2010)) and retrovirus biology (e.g. (Bueno et al., 2013)) has also been shown to be an enhancer factor for influenza virus biology (Bortz et al., 2011). Use of the PARP-1 inhibitor 3-aminobenzamide (3-AB) in PRRSV infected cells indicated that PARP-1 acted as a positive factor for virus biology, and sequential passage of the virus in the presence of 3-AB did not lead to breakthrough virus. This study also illustrated how determining the cellular interactome of a viral protein can be used to select small molecule inhibitors that can be used to target viral activity without the emergence of resistant breakthrough viruses.

## 2. Materials and methods

### 2.1. Expression of bait proteins

Single colonies from the pTriEx-PRRSV-N (in BL21(DE3)-pLysS), pHisTEV30a-MBP (His-tagged maltose binding protein, in BL21(DE3)) and pHisTEV30a-Ubc9 (SUMO conjugating enzyme, in BL21(DE3)) were cultured overnight at 37 °C until stationary phase, diluted 1:20 in 100 ml and cultured further until mid-log phase was reached (ca. $OD_{600}$ 0.5). Protein expression was induced by the addition of 0.4 mM isopropyl-β-D-thio-galactoside (IPTG). Uninduced samples served as negative controls. Bacteria were lysed in 5 ml lysis buffer (50 mM Tris (pH7.6), 300 mM NaCl, 10 mM imadaizole, 0.5% (v/v) Triton X-100, 1 mg/ml lysozyme, 10 μg/ml RNase A and 5 μg/ml DNaseI), sonicated and clarified by centrifugation. Proteins were bound to Ni-NTA beads (Qiagen) for 1 h at 4 °C, washed (50 mM Tris (pH 7.6), 300 mM NaCl, 1% (v/v) Triton X-100, 20 mM imadaizole) and their purity was checked by SDS–PAGE followed by Coomassie blue staining and immunoblotting using αHis antibodies.

### 2.2. His-tag interaction studies

Approximately $1 \times 10^7$ HEK293T cells per interaction were washed in ice-cold PBS and resuspended in 1 ml of lysis buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1% (v/v)NP40,) with 1X protease inhibitor cocktail (Roche),incubated on ice for 15 min, and clarified by centrifugation at 16,000 × *g*, 4 °C. Cellular lysates were mixed with 20 μl Ni-NTA for 30 min in order to pre-clear the sample of any proteins which would non-specifically bind to the Ni-NTA matrix. Normalized Ni-NTA-bound protein (50 μl) was mixed with the HEK293T protein lysates overnight at 4 °C. The following day the beads-protein complexes were washed three times in ice-cold PBS and eluted in 50 μl 4X LDS sample buffer (Invitrogen) with 10% DTT analyzed by silver stain and Western blot and then analyzed by mass spectrophotometry.

### 2.3. LC–MS/MS

Protein samples generated by the pulldowns were separated by one-dimensional SDS–PAGE (4–12% bis-Tris Novex mini-gel, Invitrogen). The resulting separated proteins were cut from the gel in six slices and subjected to in-gel digestion with trypsin. Trypsin digested peptides were separated using an Ultimate U3000 nanoflow LC-system (Dionex Corporation) consisting of a solvent degasser, micro and nanoflow pumps, flow control module,