RESEARCH ABSTRACT

SWINE HEALTH

Title: Defining a Novel Structural Component of Porcine Reproductive and Respiratory Syndrome Virus and its Role in Disease Pathogenesis; - **NPB #13-196**

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Scientific Abstract:

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped virus with eight recognized structural proteins encoded in the 3' end of the virus genome. Recently, nonstructural protein 2 (nsp2), part of the replicase polyprotein, was also shown to be a structural protein. Nsp2 has an extended hypervariable N-terminal domain with a recognized papain-like protease region, 4-5 predicted membrane spanning regions followed by a relatively conserved carboxyl (C)-terminus. In order to biochemically characterize the nsp2 protein independently of the virus and host cell, in vitro rabbit reticulocyte translation of nsp2 in the presence or absence of canine pancreatic microsomal membranes was used. The membranes core glycosylate proteins if a signal sequence is present, perform other post-translational modifications such as cleave signal sequences and phosphorylate residues, and protect portions of the protein that have traversed the membrane from degradation by proteases. In our experiments, we found no change in molecular weight with membranes present that would signify signal protein cleavage and core glycosylation, suggesting nsp2 is not post-translationally modified using the cell-free system. However, isoforms of nsp2 were readily seen within 30 minutes of in vitro synthesis. The insertion and topology of nsp2 was also assessed. Full-length nsp2 was found to strongly associate with membranes and, surprisingly, two additional large nsp2 isoforms of approximately 117 and 106 kDa were enriched within the membrane fraction. Membrane integration was further defined for full-length nsp2 through high-speed density fractionation, protection from protease digestion, and immunoprecipitation. The results demonstrated that nsp2 integrated into the membranes with an unexpected topology, where the N-terminal domain was located on the exterior of the membranes, corresponding to the interior of virions, and a C-terminal 15 kDa domain was located in the microsome lumen, corresponding to the extravirion space. Additional studies must be completed using the same nsp2 proteins expressed in cell culture to further probe this unexpected result.