Exploring Respiratory Syncytial Virus Fusion and Matrix Protein Interactions in Membranes Using Recombinant Proteins and Nanodiscs

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Introduction

Background:
Respiratory syncytial virus (RSV) belongs to the *Paramyxoviridae* family and is the leading cause of severe viral respiratory disease in young children, the elderly and immunocompromised individuals. RSV infects predominantly airway epithelial cells of the respiratory tract, where it exhibits directional assembly and budding from the apical surface of polarized cells, generating filamentous structures.

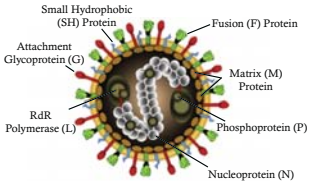


Figure 1: Diagram of RSV virion structure with structural features and viral proteins indicated (1).

Objective:
Given the hypothesized interaction between RSV matrix (M) protein and fusion (F) protein, it has been difficult to detect this through commonly used molecular biology techniques. We sought to develop innovative methods to examine the interaction between these viral proteins utilizing site directed mutagenesis, nanodiscs and Förster resonance energy transfer (FRET). Data obtained through these various assays provides insight into the mechanism controlling RSV viral egress, leading towards the development of novel disease interventions.

Hypothesis:
We hypothesized that RSV M and RSV F have a transient interaction that mediates assembly of the viral proteins into filamentous structures prior to budding and release from epithelial cells.

Experimental Approach

1. Generation of RSV F CT and F CT mutants for nanodisc assembly

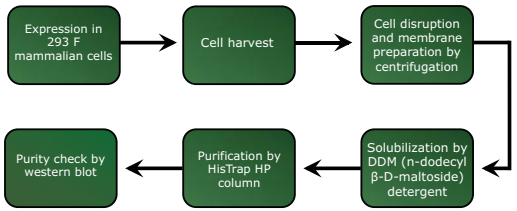


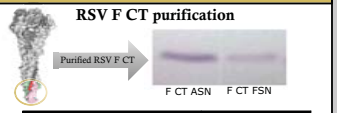
Figure 2: Overview for membrane protein isolation and purification.

2. Site directed mutagenesis of the RSV M and filament formation
   - ✧ Transfection of African green monkey kidney cell (i.e. vero cells) and and human larynx cell (i.e. HEp-2 cells)
   - ✧ Immunofluorescence assay (IFA) staining against RSV F, N, M, nuclei, and actin
   - ✧ Confocal microscopy (LSM710) at 63x magnification

3. Enzyme-linked immunosorbent assay (ELISA) examine interactions between RSV M and F CT
   - ✧ Purified RSV M protein interactions using biotin-labeled F CT peptides

Results

Figure 3: Western blot analysis was performed on purified wildtype RSV F CT (FSN) and mutant FCT (ASN) utilizing rabbit anti-RSV F CT polyclonal antibody, detected using goat anti-rabbit-AP secondary and visualized with chromogenic substrate (BCIP/NBT). Concentration was determined via spectrometry (2).



	F CT Sequence	Final [(mg/mL)]
F CT FSN	KARSTPVTLSKDQLSGINNAFSN	.081
F CT ASN	KARSTPVTLSKDQLSGINNAASN	.198

Attenuated filament formation in epithelial cells

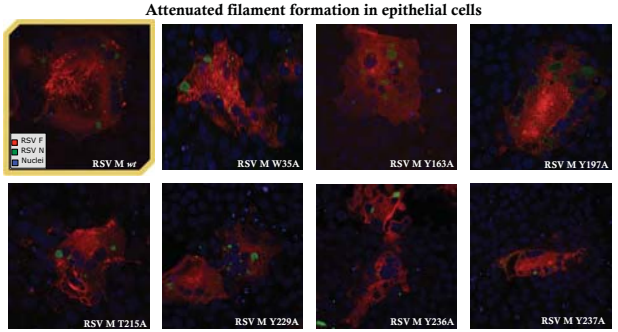


Figure 4: Confocal images of RSV M and RSV M point mutants in transiently transfected vero cells at 72 hour timepoint. RSV F (red), N (green), and nuclei (blue) were IFA stained.

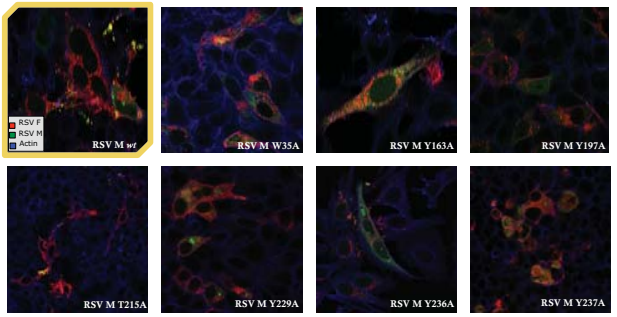


Figure 5. Confocal images of RSV M and RSV M point mutations, where HEp-2 cells were transiently transfected for 48 hours and the RSV M (green), RSV F (red), and actin (blue) proteins were IFA stained.

Terminal RSV F CT Phe is critical for interaction with matrix protein

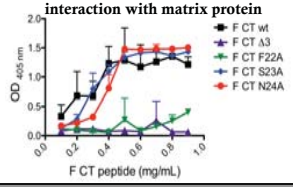


Figure 6: ELISA was used to examine the interaction between RSV F CT biotin-labeled peptides and RSV M protein. Each plate was coated with purified RSV M protein at a concentration of 2 µg/mL and probed with (1.0 – 0.1 mg/mL) of each indicated F CT peptide. Streptavidin alkaline phosphatase was used for detection of biotinylated molecules. Absorbance (OD) was read at 405 nm.

Conclusions

- ✧ Completed and verified integral membrane protein purification of wildtype RSV F CT (FSN) and the mutated form of F CT (ASN) for assembly on nanodisc.
- ✧ Site directed mutagenesis of RSV M shows that it is required for filament formation in both vero and HEp-2 epithelial cell lines. All single point mutations of RSV M lead to either attenuated filament formation or loss of filaments.
- ✧ Demonstrated that RSV M and the cytoplasmic tail of F interact utilizing an ELISA based approach, where the terminal phenylalanine (Phe) of the F CT is critical for the observed interaction.

Future Work

Future work includes assembling the RSV F CT protein on a nanodisc platform, using a more biologically relevant approach to demonstrate the interaction between the RSV F CT and RSV M. This information, along with solving the RSV M and F CT crystal structure will allow us to understanding how viral proteins interact within the host. Ultimately giving insight into the mechanisms for the development of antiviral therapeutics.

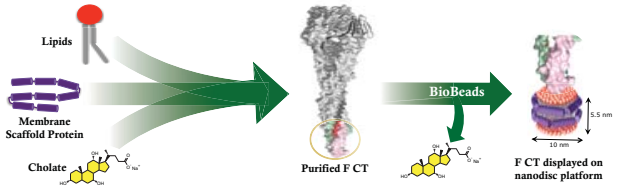


Figure 7: Nanodisc assembly occurs by mixing membrane scaffold proteins with cholate-solubilized phospholipids and detergent solubilized purified F CT proteins. BioBeads are then used to remove all detergents, resulting in a protein displayed on a discoidal phospholipid bilayer (2,3).

Acknowledgments

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