Functional implications of natural polymorphisms in the p10 protein of avian reovirus

Yang Yang, Sara Lynn Purcell, Lisanework Ayalew

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada

Introduction

- Avian reoviruses (ARVs) are non-enveloped viruses with segmented double stranded RNA genomes
- Infection causes tenosynovitis/arthritis in poultry
- ARV disease outbreaks are associated with significant economic losses
- To date, six different genotypic cluster groups are identified
- The S1 genome segment which encodes for p10, p17 and Sigma-C proteins is highly variable
- The p10 protein, encoded by the S1 gene segment, is a non-structural transmembrane protein critical for cell-cell fusion and viral dissemination
- There is lack of information regarding the impact of natural polymorphisms in ARV genomes in viral protein function.

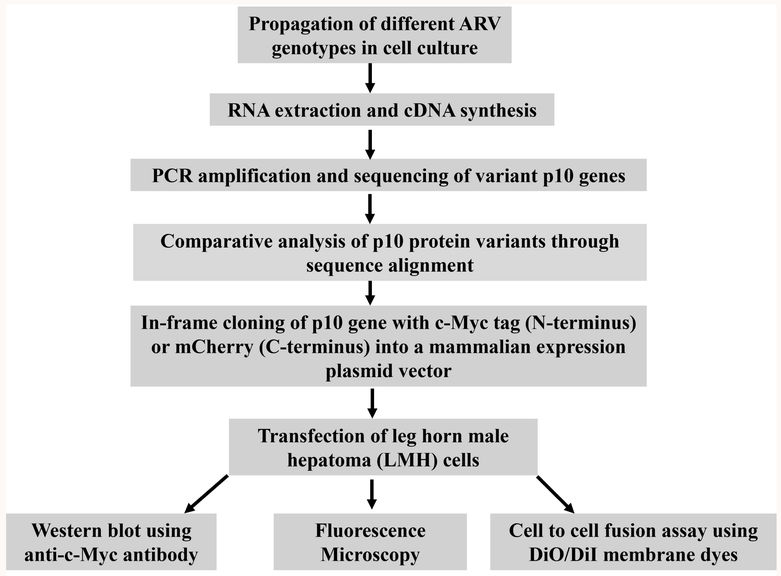
Hypothesis and Objectives

Hypothesis: Natural polymorphisms could alter structure, conformation, and function of p10 protein which leads to differences in p10 - cellular protein-protein interaction networks and protein functionality.

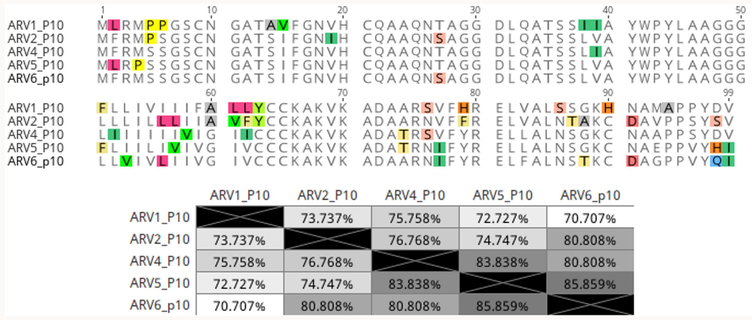
Objectives:

1. Cloning and expression of variant p10 proteins and analysis of fusogenic activities
2. Analysis of cellular protein networks which interact with variant p10 proteins
3. Mapping of functional domains of variant p10 proteins

Materials and Methods



Results



	ARV1_P10	ARV2_P10	ARV4_P10	ARV5_P10	ARV6_p10
ARV1_P10		73.737%	75.758%	72.727%	70.707%
ARV2_P10	73.737%		76.768%	74.747%	80.808%
ARV4_P10	75.758%	76.768%		83.838%	80.808%
ARV5_P10	72.727%	74.747%	83.838%		85.859%
ARV6_p10	70.707%	80.808%	80.808%	85.859%	

Figure 1. Comparison of p10 protein amino acid sequences from ARV strains representing different genotyping groups. Sequence alignment was performed using the Clustal Omega algorithm in Geneious Prime software(Dotmatics).

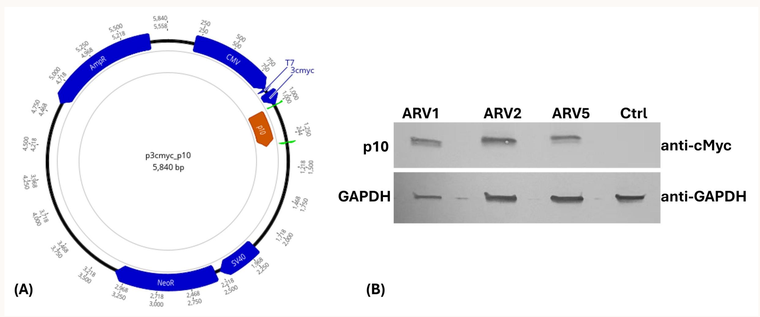


Figure 2. Cloning and expression of the p10 protein from various ARV genotypic groups Leghorn Male Hepatoma (LMH) cells . (A) Schematic representation of the cloning strategy. (B) Western blot analysis of p10 protein expression.

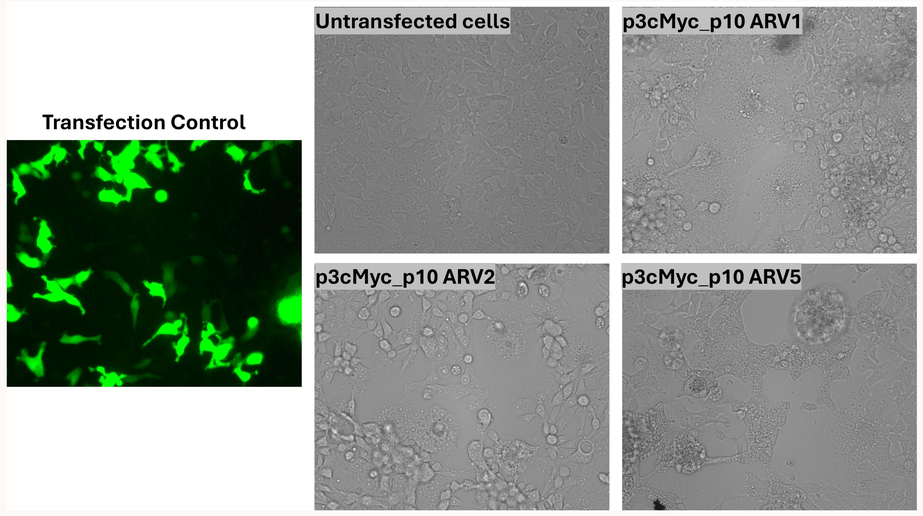


Figure 3. Induction of cell–cell fusion (syncytium formation) by p10 protein variants. LMH cells were transfected with plasmid DNAs expressing different ARV p10 variants and examined by light microscopy. Cells transfected with a green fluorescent protein (GFP)-expressing plasmid DNA served as a transfection control.

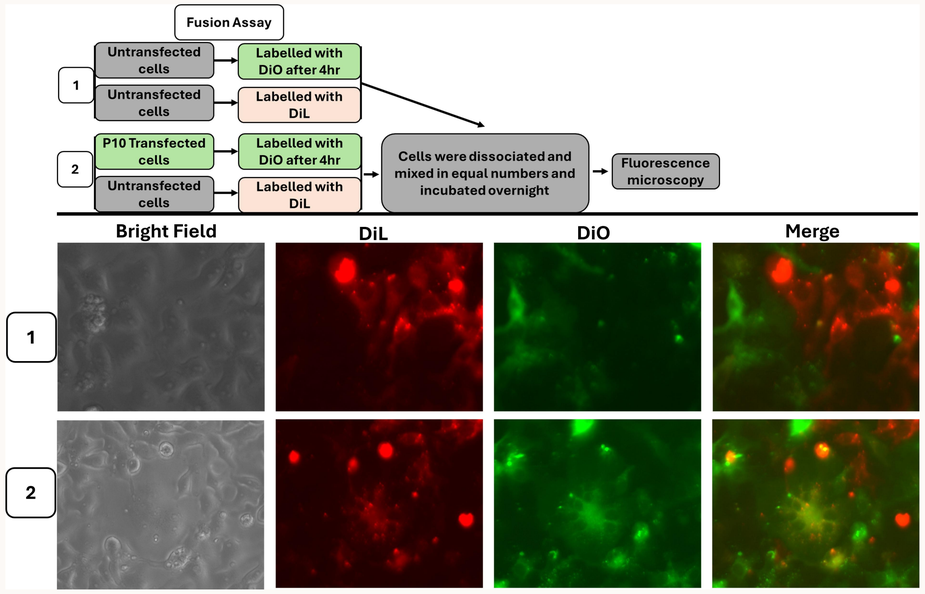


Figure 4. Fusion assay to assess p10-mediated cell–cell fusion. LMH cells were transfected with pcMyc_p10 plasmid DNA. Four hours post-transfection, cells were labeled with DiO (a green membrane dye), dissociated, and mixed in equal proportions with untransfected LMH cells labeled with DiL (a red membrane dye). Cell mixtures were examined under a fluorescence microscope to assess fusion. DiO- and DiL-labeled untransfected cells mixed in equal proportions served as negative controls.

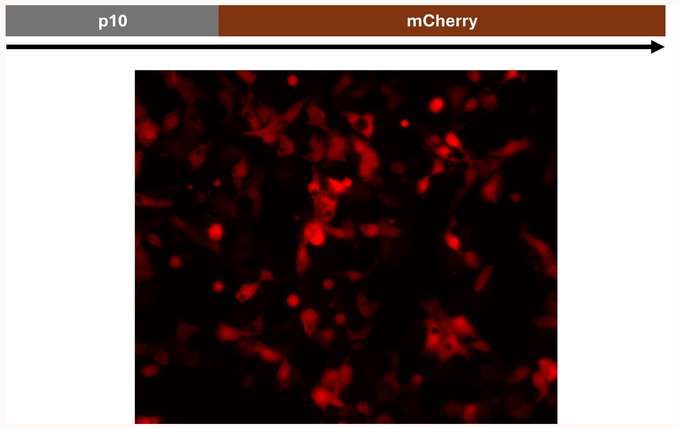


Figure 5. Cloning and expression of p10 variant proteins fused with mCherry at the C-terminus. C-terminal fusion with mCherry abolished the fusogenic activity of the p10 protein in LMH cells.

Conclusions

- p10 proteins are highly variable between various genotypic cluster groups of ARV.
- All the variant p10 proteins were capable of inducing cell to cell fusion.
- p10 protein of ARV2 appeared to have less fusogenic activity than ARV1 and ARV5.
- The integrity of the C-terminus of p10 protein is required for its fusogenic activity.

Work in progress and future directions

Understanding variant p10 protein interactions with the cellular machinery through:

- Transcriptomics to measure host gene expression changes in response to ARV infection and p10 expression.
- Extraction of membrane proteins from p10 expressing plasmid DNA transfected and ARV infected cells followed by mass spectrometry analysis.
- Co-immunoprecipitation of proteins interacting with p10 using cells transfected with variant p10-expressing plasmid or infected with ARV variants, followed by mass spectrometry analysis.
- Mapping of functional domains of the p10 protein involved in interactions with key cellular proteins.

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