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Bioinformatic analysis of the avian nucleolar proteome

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Abstract

The following work was undertaken to elucidate the avian nucleolar proteome as a functional dataset and to explore how the nucleolus responds to infection with avian infectious bronchitis virus (IBV).

This study presents the first solution of the avian nucleolar proteome and begins to explore the network pathways involved during infection with IBV.

Introduction

Avian infectious bronchitis virus (IBV) is a member of the *Coronaviridae,* a family of enveloped viruses with single-stranded positive-sense RNA genomes. It causes extensive damage to the avian respiratory tract and represents a substantial economic cost to the poultry industry.

The DF-1 cell line used for this work is a spontaneously immortalised continuous cell line of chicken embryo fibroblasts with normal fibroblast morphology, which rapidly proliferates.

The Nucleolus

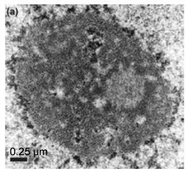
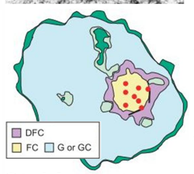


Figure 1 The nucleolus is a subnuclear structure, comprising three discrete subregions: the fibrillar centre (FC), dense fibrillar component (DFC) and the granular component (GC). (Image adapted from Thiry & Lafontaine (2005) and is representative of a human nucleolus)

It has recently come to light that the nucleolus plays a role outside its traditional function in ribosome biogenesis.



The nucleolus is a target for cancer and infectious disease, with many viral proteins known to localise to this structure. It has been observed that the nucleolus can change morphology in virus-infected cells and this may correspond with a change in proteome.

Stable Isotope Labelling with Amino Acids in Cell Culture (SILAC) is a method for identification and quantification of complex protein mixtures, when coupled with mass spectrometry (MS) and is rapidly advancing the field of expression proteomics.

This method can be applied to populations of cells pre- and post-viral infection, and the data used to ascertain significant changes in the nucleolar proteome in response to infection.

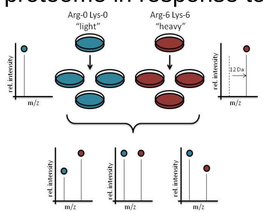


Figure 2 Heavy arginine and lysine isotopes are incorporated into proteins and cause a shift upwards in molecular weight of the "heavy" labelled population's proteins. Ratios of heavy:light labelled proteins allows the number-fold increase or decrease in protein abundance to be identified.

This is the premise for the use of SILAC in this work.

SILAC, cellular fractionation and LC-MS/MS of mock- and virus-infected cells → Bioinformatic analysis of mass spec data *in silico* → Output tables of nucleolar proteome and proteins impacted by viral infection → Immunofluorescence confirmation of SILAC data → Paper submitted to *Proteomics*: solution of the avian nucleolar proteome

Results

Nucleolar Proteome

- MS identified **937** chicken proteins and **534** human-equivalent proteins in the DF-1 nucleolar fraction.
- **378** of the human proteins were then found to be equivalent to proteins in the human nucleolar proteome database (NOPdb) @www.lamondlab.com
- Of these 378 nucleolar proteins, **109** were within the set cut-off range of FPR <0.1, with more than a two-fold change in abundance in IBV infected cells.

Cellular and Molecular Functions of the Avian Nucleolar Proteome

The functions of the proteins identified in the nucleolar proteome were identified and are displayed in the following pie chart.

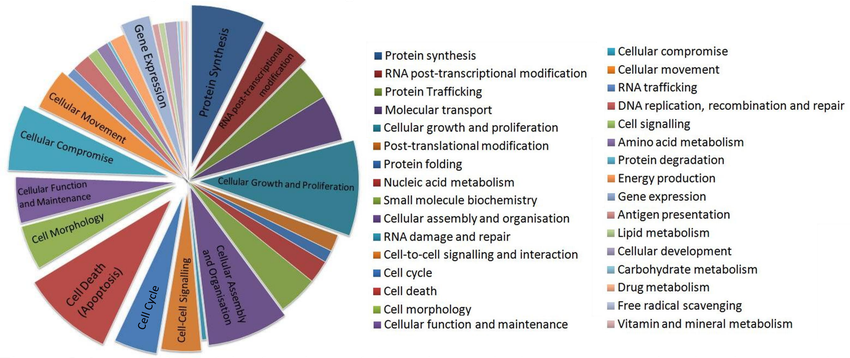


Figure 3 An overview of the functions of the avian nucleolar proteome, showing the proportion of proteins involved in different aspects of cellular function. Prominent functional groupings are labelled.

Network and Pathway Analyses

Major linked functions of the nucleolar proteome include:

- Protein synthesis, gene expression, RNA post-transcriptional modification, DNA replication, recombination and repair and cellular assembly and organisation.
- Cellular assembly and organisation.
- Cell growth and proliferation linked to protein synthesis.

Pathways affected by IBV infection include:

- ILK signaling pathway
- Actin cytoskeletal signaling pathway
- 14-3-3 signaling pathway

Changes in the regulation of these pathways is known to lead to disruption of cell cycle control, affecting proliferation, morphology and apoptotic mechanisms.

Conclusions

- This work presents the first solution of the avian nucleolar proteome.
- In general there is an increase in nucleolar proteins in IBV-infected cells.
- Proteins involved in aspects of cell cycle other than ribosome biogenesis were also present in the nucleolus.
- Cell cycle aberrations during infection with IBV were predicted by bioinformatic analysis.
- This study demonstrates the usefulness of SILAC coupled with LC-MS/MS in studying changes in organelle proteomes during virus infection.

Future work

The avian nucleolar proteome is still far from complete.

There are ways to improve coverage in the SILAC-MS data and investigation using primary cells lines would increase the reliability of our dataset.

Further annotation of the chicken genome will allow better identification of the proteins identified by MS so that the full raw data set can be used, instead of the limited human-compared set.

Understanding cellular responses to IBV infection provides insights for potential treatment of infection to limit economic impact.

Key references

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Acknowledgments

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