BBSRC Strategic Research Priority
Food Security
- Farm animal health, Fisheries and bees

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PhD project title:
Characterising mechanisms that control Marek’s Disease Virus genome release from telomeres during reactivation from latency.

University of Registration: Leicester

Project outline
1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns)

Infection by Marek’s Disease Virus (MDV) is followed by latency, T-cell lymphomas and reactivation. It has significant deleterious effects on chicken health and welfare, with an annual estimated loss close to $2 billion to the global poultry meat and egg production industries. MDV infection is via inhalation of MDV virions. Cytolytic infection in the lung epithelium, macrophages and B-cells is followed by establishment of latent infection in T-cells, via MDV integration into chicken telomeres. Neoplastic transformation occurs in CD4+ T-cells harbouring multiple integrated copies of the MDV genome. The aggressive lymphomas result in high levels of chicken mortality.

The presence of (TTAGGG)n repeats within some herpesvirus genomes, including MDV and human herpesvirus 6 (HHV-6), suggests that telomeric integration is an advantageous, conserved latency mechanism. Once integrated, the viral genome is epigenetically silenced and maintained as part of the nuclear genome. For telomeric integration to be an effective latent state there must be a mechanism to release the viral genome from the telomere and to reverse the silencing. We hypothesise that excision of integrated viral genomes is dependent on processes, such as telomere replication and t-loop formation. We also propose that epigenetic features of telomeres, for example the expression of non-coding telomeric RNA (TERRA) may play important roles during latency.

Telomeres are the essential capping structures of linear chromosomes. Capping is achieved through binding to a conserved multi-protein complex known as Shelterin, which facilitates the formation of looped structures (t-loops) and regulates access by telomerase and other protein complexes. T-loops are formed by invasion of the single stranded telomeric 3’ overhang into the upstream duplex DNA and
formation of a D-loop at the point of insertion. T-loops are dynamic structures that are unravelling during S-phase or alternatively they can be cleaved releasing circular DNA molecules. We have proposed a model in which a chromosomally-integrated HHV-6 genome can be excised from a telomere by t-loop formation within the viral genome and cleavage. Now we need to establish whether the mechanism is conserved and facilitates excision of integrated MDV (iMDV) from chicken telomeres. Insight into the mechanism and its regulation may offer novel ways to control MDV reactivation.

As stated, telomeric DNA is transcribed into non-coding \((\text{UUAGGG})_n\) telomeric RNA (TERRA). TERRA levels peak during S-phase, suggesting a role in telomere replication, among other roles. Telomere stability also requires ATRX/DAXX-dependent deposition of the H3.3 histone variant in a replication-independent manner. These normal features of telomeres may play roles in silencing iMDV genomes and maintenance of latency. Furthermore, disturbance of the chromatin organisation or expression of TERRA has the potential to cause localised telomere instability that could contribute to release of the iMDV genome and tumorigenesis.

**The aim** of this project is to conduct comparative analysis of the mechanisms that facilitate HHV-6 and MDV excision from telomeres in order to characterise factors that control the transition from latency to reactivation.

**Objectives:**

1) Identify genes that influence release of iMDV and HHV-6 genomes from telomeres.
2) Determine whether the presence of a fully length herpesvirus genome (MDV or HHV-6) causes replication stress and therefore telomere instability during S-phase.
3) Establish whether TERRA transcripts and other telomeric epigenetic features play roles in iMDV genome stability, latency and reactivation.

The project offers the opportunity to conduct research within a world-renowned Genetics Genome Biology Department (University of Leicester) and backed by the expertise of staff and the facilities available in the Pirbright Institute.

References:


**Techniques that will be undertaken during the project**

- Complex PCR to analyse telomeres and telomere stability;
- Sequence analysis of MDV and chicken genes and promoters – Sanger and next generation technologies; Sequencing using PacBio technology. Bioinformatic analyses associated with the various sequencing technologies
- Copy number analysis of MDV in chicken cells using droplet digital PCR.
- Fluorescent in situ hybridisation to characterise telomere stability and immunofluorescence to detect proteins colocalising with telomeres and MDV
• Chicken gene expression analysis using a variety of established methods
• Mutation of selected chicken genes using CRISPR technology to measure effect on iMDV excision and reactivation