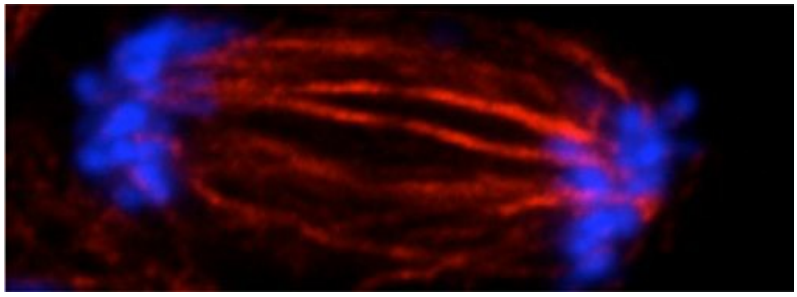
Human Papillomavirus Persistence Research Group



Group leader **Dr Jo Parish (/staff/profiles/cancer/parish-jo.aspx)**

Overview

HPV infection can cause benign lesions or warts, and several malignancies of the epidermal layer including cervical cancer. The majority of sexually active adults will become infected with a genital HPV type at least once in their lives. Although HPV infection causes carcinoma in a relatively small number of infected individuals, 99.7% of cervical carcinomas are associated with HPV infection and each year 240,000 cervical cancer related deaths are reported. Work in the Parish lab is focused on the study of the molecular interactions required for HPV persistence and completion of the viral life cycle.

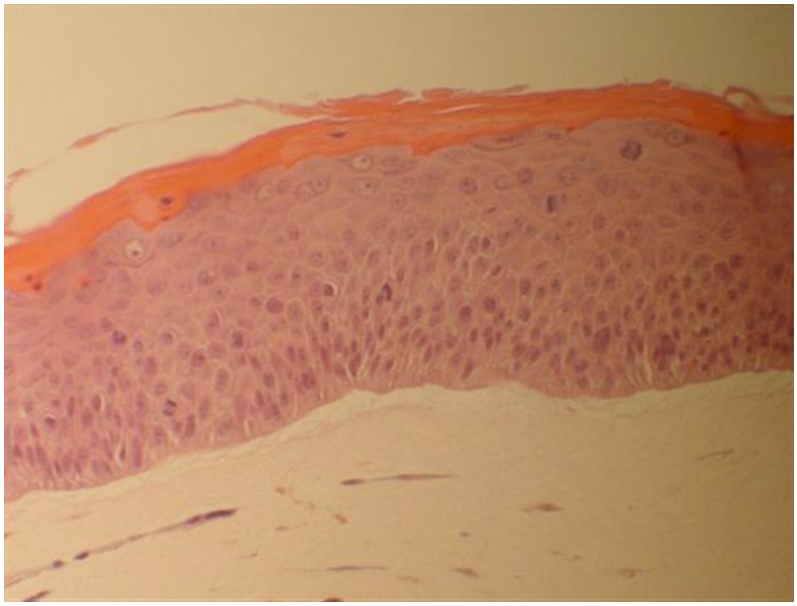
Our research group

The Human Papillomavirus Persistence Research Group studies several novel virus host interactions that are important for the persistence of HPV infections and completion of the virus life cycle. We have a particular interest in protein-protein interactions and have isolated novel virus-host interactions mediated by the HPV early protein, E2.

Our work is primarily cell biology based and we use a variety of techniques to study the effect of HPV gene expression on host cell pathways. We characterise protein-protein interactions *in vivo* using co-immunoprecipitations and high-resolution immunofluorescent microscopy combined with fluorescence resonance energy transfer (FRET). We also use a variety of biophysical techniques including fluorescence polarisation and BIAcore analysis to characterise protein-protein interactions *in vitro*.

We are also interested in the interaction of host cell proteins with the HPV genome. These interactions are studied *in vivo* in cells that maintain episomal HPV genomes by chromatin immunoprecipitation (ChIP) assay. The effect of viral integration on the association of host cell proteins with the HPV genome and the regulation of host cell gene expression is also an area of interest.

Characterised virus-host interactions are studied in the context of the virus life cycle using the organotypic raft culture model system. Mutation of viral proteins such that they no longer associate with the target protein of interest is important for these studies. Once mutations have been introduced and characterised, they are engineered into the complete HPV genome, which is transfected into primary keratinocytes. This work is in collaboration with **Dr Sally Roberts (/staff/profiles/cancer/roberts-sally.aspx)** .



Above: Hematoxylin and eosin staining of formalin-fixed and paraffin embedded slice of an organotypic raft of human primary foreskin keratinocytes maintaining episomal HPV18 genomes.

Current projects

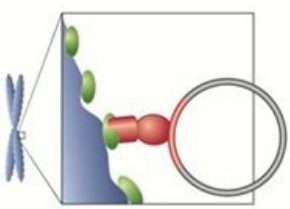
Open all sections

The HPV E2 protein targets several cellular proteins during DNA replication and mitosis to facilitate viral DNA replication and persistence. Work is on-going to characterise these virus-host interactions and decipher their importance in the HPV life cycle.

Characterisation of the functional interactions facilitated by the HPV E2 proteins that are required for viral persistence.

Work in the Parish laboratory is primarily focused on how HPV genomes are passed to daughter cells during mitotic division. To ensure the passage of viral genomes to new cells, papillomaviruses hijack some of the mechanisms that exist in host cells required the maintenance of genome stability. In particular, the HPV E2 protein targets several cellular proteins during DNA replication and mitosis to facilitate attachment of genomes to mitotic chromosomes (Figure 1). Work is on going to characterise these virus-host interactions and the cellular pathways hijacked by HPV and decipher their importance in the HPV life cycle. This project is funded by the *Royal Society*.

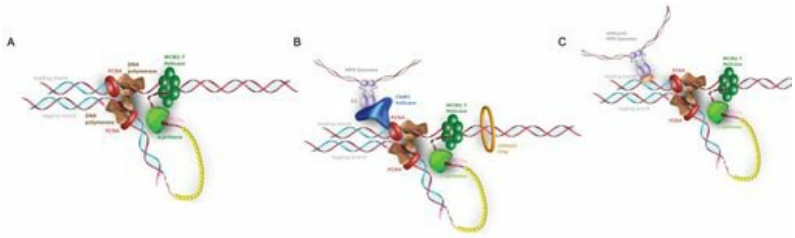
Right: Mechanism of viral genome tethering during mitosis. Many episomally maintained DNA viruses tether genomes to host cell chromosomes during mitosis. In general, a virally encoded DNA-binding protein (red; here shown as HPV E2) associates simultaneously with the viral genome (circle) and host cell chromatin (blue) or chromatin-bound cellular protein(s) (green).



Functional analysis of the ChlR1 DNA helicase in HPV and host cell DNA replication.

Work is on-going in the Parish lab to characterise to function of the ChlR1 DNA helicase in host cell DNA replication. Using a variety of methods including the DNA fibre technique, immunofluorescent microscopy, cell cycle analysis and sister chromatid cohesion analysis, we are able to study the function of this DNA helicase in the maintenance of host cell genome stability.

We are also interested in how ChlR1 facilitates the persistence of HPV episomes. Using FRET analysis we have shown that E2 directly targets ChlR1 during DNA replication. This has lead us to propose the model shown in figure 2. This work is funded by *Medical Research Scotland* and *The Royal Society*.



Above: Targeting of ChlR1 by HPV E2 during DNA replication to ensure viral persistence. (A) Cellular DNA replication fork movement from left to right. (B) Replication pauses as a cohesin ring is encountered promoting recruitment of ChlR1, E2 and viral DNA. (C) ChlR1 promotes re-initiation of DNA replication and leaves the complex. HPV E2 and viral DNA stay bound to the chromatin.

Characterisation of DNA binding proteins recruited to the HPV genome to control viral gene expression.

Using chromatin immunoprecipitation approaches we have identified several novel host cell proteins that are recruited to HPV genomes. We are currently characterising these interactions and their importance in the control of viral gene expression and/or replication. This project is funded *Cancer Research UK* and *Tenovus Scotland*.

The study of novel interactions between the HPV E2 and host cell proteins that alter sub cellular vesicle trafficking within infected cells.

We have recently identified a novel binding partner of E2 that is important in the regulation of sub-cellular trafficking. Preliminary studies suggest that E2 alters the function of the protein and therefore alters vesicle trafficking within infected cells. We are currently characterising this interaction and its role in the completion of the HPV life cycle.

Recent publications

- **Parish J. L.,** Rosa J., Wang X., Lahti J., Doxsey S. J., Androphy E. J. The DNA helicase ChlR1 is required for sister chromatid cohesion in mammalian cells. (2006) *Journal of Cell Science.* 119, 4857-4865.
- **Parish J. L.,** Bean A.M., Park R.B., Androphy E.J. ChlR1 is required for loading papillomavirus E2 onto mitotic chromosomes and viral genome maintenance. (2006) *Molecular Cell.* 24(6), 70-76.
- Feeney K.M. and **Parish J. L.** Targeting mitotic chromosomes: a conserved mechanism to ensure viral genome persistence. (2009) *Proceedings of the Royal Society B: Biological Sciences.* 276 (1662):1535-44.
- Jolly C. E., Gray L. J., **Parish J. L.**, Lain S. and Herrington C. S. Leptomycin B Induces Apoptosis in Cells Containing the Whole HPV 16 Genome. (2009) *The International Journal of Oncology.* 35:649-656.
- Feeney K. M., Saade A., Okrasa K. and **Parish J. L**. *In vivo* analysis of the cell cycle dependent association of the bovine papillomavirus E2 protein and ChlR1 2011. *Virology*. 414:1-9.

Staff

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