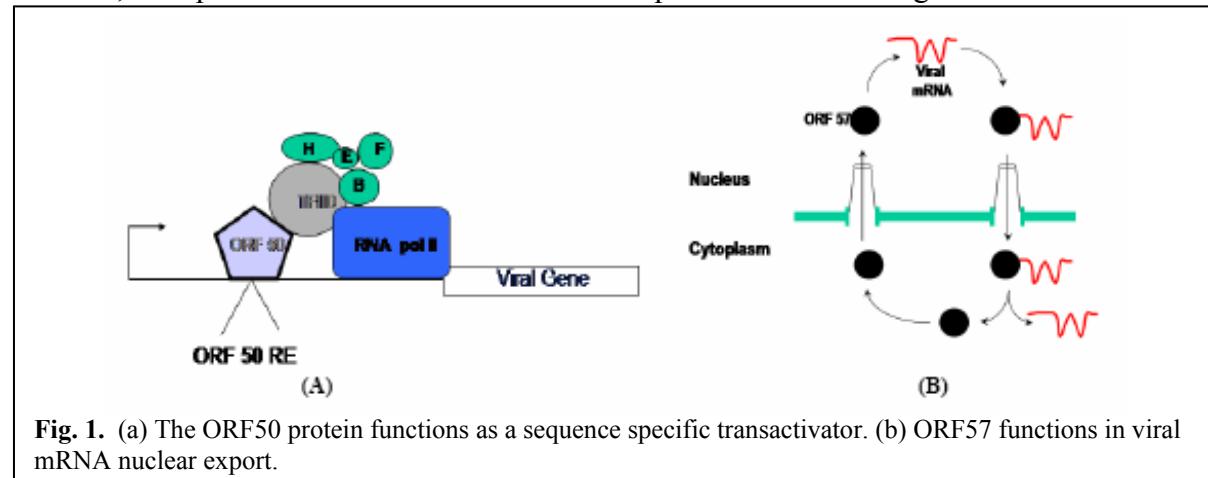


Herpes viral-host cell interactions which regulate viral gene expression

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Gamma-2 herpes viruses are an increasingly important sub-family of herpes viruses with oncogenic potential, particularly as a result of the identification of the first human gamma-2 herpes virus, Kaposi's sarcoma-associated herpes virus (KSHV). KSHV has rapidly become the focus of intensive research as epidemiological studies suggest it is the etiologic agent of Kaposi's sarcoma, the most common AIDS-related malignancy. In addition, the presence of the virus has been detected in a variety of lympho-proliferative disorders including primary effusion lymphoma and multicentric Castleman's disease. However at present, analysis of KSHV gene function is hampered by the lack of a permissive cell culture system. Therefore, the ability to easily grow and manipulate the prototype gamma-2 herpes virus, HVS, *in vitro* has made this virus an attractive model for the analysis of gamma-2 herpes viruses in general. Therefore, we have a major research focus investigating the virus-host cell interactions which regulate the early events in gamma-2 herpes virus replication cycles, in particular HVS and more recently KSHV.

The interaction of the major transcription control protein, ORF 50 and viral promoters. The ORF 50 protein is the latent-lytic switch gene in gamma-herpes viruses and transactivates delayed-early gene expression. It functions as a sequence specific transactivator, binding to an A/T rich ORF 50 response element with DE promoters (Fig. 1a). We have demonstrated that ORF 50 contains a DNA binding domain that has homology to an AT-hook DNA binding motif. The AT-hook is a small DNA-binding protein motif that was first described in the non-histone chromosomal protein HMGA, and allows binding to the minor groove of short stretches of AT-rich DNA. The AT-hook has a core consensus sequence of Pro-Arg-Gly-Arg-Pro (with R-G-R-P being invariant), flanked on either side by a number of positively charged lysine/arginine residues. The core of the AT-hook peptide motif is highly conserved in evolution from bacteria to humans and is found in one or more copies in a large number of other, HMGA proteins, many of which are transcription factors or components of chromatin remodelling complexes. Deletion analysis of this domain reduces ORF 50-mediated transactivation of the DE ORF 6 and ORF 57 promoters by 100% and 90%, respectively. Furthermore, gel retardation experiments demonstrated that the AT-hook motif was required for binding the ORF 50 response element in the promoters of DE genes. Single site-directed mutagenesis of the AT-hook revealed that mutation of the glycine residue at position 408 to an alanine reduced ORF 50 transactivation of the ORF 57 promoter by 40%. Moreover, mutation of multiple basic residues in conjunction with the glycine residue within the core element of the AT-Hook abolishes ORF 50-mediated transactivation. In addition, the p50GFP Δ AT-hook mutant was capable of functioning as a *trans*-dominant



mutant leading to a reduction in virus production of approximately 50% compared to wild-type ORF 50. We are presently characterising these protein-DNA interactions using surface plasmon resonance (SPR) and fluorescence resonance energy transfer (FRET).

The interaction of the nucleocytoplasmic shuttle protein, ORF 57 and viral mRNA.

The ORF 57 protein encodes a nuclear cytoplasmic shuttle protein which mediates the nuclear export of viral mRNAs. We have recent analysis demonstrating that ORF 57 has the ability to bind viral RNA, shuttle between the nucleus and cytoplasm and is required for efficient nuclear export of viral transcripts (Fig. 1b). Moreover, we have shown that ORF57 shuttles between the nucleus and cytoplasm in an CRM-1 independent manner. ORF 57 interacts with the mRNA export factor REF and two other components of the exon-junction complex, Y14 and Magoh. The association of ORF57 with REF stimulates recruitment of the cellular mRNA export factor TAP, and HVS infection triggers the relocalisation of REF and TAP from the nuclear speckles to several large clumps within the cell. Using a dominant negative form of TAP and RNA interference to deplete TAP, we show that it is essential for bulk mRNA export in mammalian cells and is required for ORF57 mediated viral RNA export. Furthermore, we show that disruption of TAP reduces viral replication. These data indicate that γ -2 herpes viruses utilise ORF57 to recruit components of the exon-junction complex and subsequently TAP to promote viral RNA export via the cellular mRNA export pathway. We now aim to analyse the domains required for these interactions in more detail using structural analysis.

Publications.

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Collaborators.

Stuart Wilson, University of Sheffield.

Funding.

This work has been funded in parts by the BBSRC, MRC, YCR and Royal Society.