Identification of the ribonucleoprotein complex required for efficient viral RNA processing in oncogenic herpesviruses

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Introduction

Post-transcriptional events which regulate mRNA biogenesis are central to the regulation of gene expression. As a consequence, cells have evolved a 'gene-expression production line' that encompasses the routing of a nascent transcript through multimeric mRNA–protein complexes that mediate its splicing, polyadenylation, nuclear export and translation. Of these events it has become clear that splicing is particularly important for mRNA nuclear export, as recruitment of multiprotein complexes required for mRNA export are bound to mRNA in a splicing dependent manner. Two multiple protein complexes, namely, hTREX and the EJC bind at separate locations on spliced mRNA. hTREX, which comprises Aly, UAP56 and the multiprotein ThoI complex, is recruited exclusively to the 5' end of the first exon, providing 5'-polarity and therefore directionality observed in mRNA export.

However, in contrast to the majority of mammalian genes, analysis of herpesvirus genomes has highlighted that most lytically expressed viral genes lack introns. Herpesviruses replicate in the nucleus of the host mammalian cell, and therefore require their intronless mRNAs to be exported out of the nucleus to allow viral mRNA translation in the cytoplasm. This therefore leads to an intriguing question concerning the mechanism by which the viral intronless mRNAs are exported out of the nucleus in the absence of splicing. To circumvent this problem, and to facilitate viral mRNA export, γ-2 herpes viruses encode the ORF 57 protein. ORF 57 interacts with Aly, binds viral RNA, shuttles between the nucleus and the cytoplasm and promotes the nuclear export of viral mRNA.

Results

We are currently investigating how an intronless viral mRNP is assembled in KSHV and what role ORF57 plays in that process. We have shown that ORF57 interacts with hTREX and is essential for the recruitment of hTREX onto intronless viral mRNA transcripts. Importantly, ORF57 does not recruit the EJC to intronless viral transcripts. Moreover, we are currently determining how ORF 57 recognises the viral mRNA and allows recruitment of hTREX. This is the first system that has distinguished between hTREX and EJC *in vivo* and demonstrates that recruitment of hTREX alone to mRNA transcripts is sufficient for their nuclear export. Therefore, we believe this viral system is an exciting model to further study mRNA export mechanisms. We propose a model for herpesvirus mRNA export, whereby ORF57 mimics splicing in order to recruit the mRNA export machinery to intronless viral mRNAs.

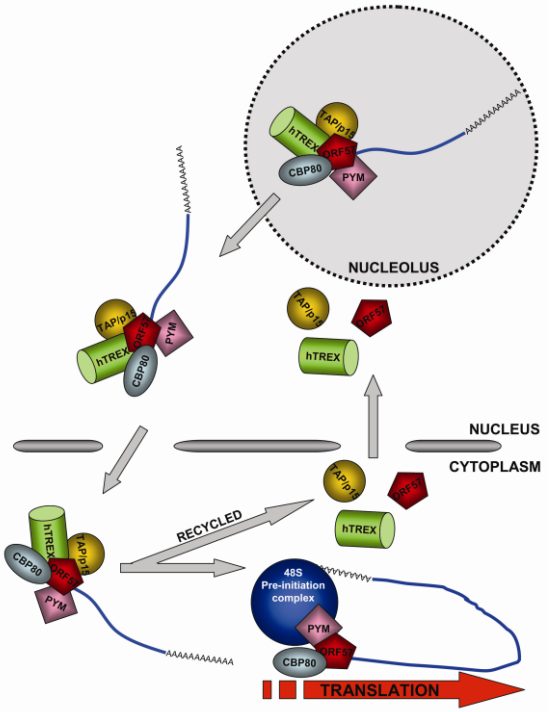

Figure 1: KSHV ORF57 mimics splicing to recruit the necessary nuclear export factors onto viral intronless transcripts and also mimics an EJC to enhance their translation.

We are now determining the structure of the interaction interface at atomic-resolution

between ORF57 homologues and the hTREX proteins, such as Aly, In collaboration with Dr Alexander Golovanov (University of Manchester) and Professr Stuart Wilson (University of Sheffield). This will provide a detailed comparison of the binding interfaces between ORF57 homologues and Aly using solution-state NMR. The regions of HSV ICP27 and HVS ORF57 involved in binding by Aly have been mapped as residues 104-112 and 103-120, respectively. We have identified the pattern of residues critical for Aly recognition, common to both ICP27 and ORF57. The importance of the key amino acid residues within these binding sites was confirmed by site-directed mutagenesis. The functional significance of the ORF57-REF/Aly interaction was also probed using an *ex vivo* cytoplasmic viral mRNA accumulation assay and this revealed that mutants that reduce the protein-protein interaction dramatically decrease the ability of ORF57 to mediate the nuclear export of intronless viral mRNA. Together these data precisely map amino acid residues responsible for the direct interactions between viral adaptors and cellular REF/Aly and provide the first molecular details of how herpes viruses access the cellular mRNA export pathway. Future work will utilise these identified binding interfaces as possible new drug targets, to be used in the future for anti-viral drug design efforts, for the prevention or treatment of KSHV-related malignancies using rational-based drug design approaches.

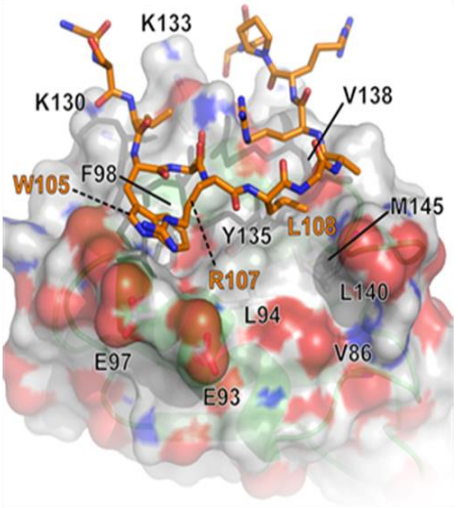

Figure 2: ORF57 homologue (HSV ICP27) recognizes and specifically binds to the surface of the RRM domain of cellular export factor REF/Aly

Publications

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