What inspired you to pursue your research on the oncogenic virus, Kaposi’s sarcoma-associated herpesvirus (KSHV)?

I’ve always had a strong interest in the study of DNA and RNA, and in particular the protein complexes that manipulate these essential nucleic acids. My PhD focused on the DNA repair pathways of a model archaeal organism. However, progressing onto my postdoctoral studies I wanted to move my research into the field of human disease, while maintaining my interest in the processing of nucleic acids. Herpesviruses were therefore an excellent choice as they have been used as model organisms for understanding the processing of RNA in humans, particularly by the Whitehouse Lab at the University of Leeds, UK.

Can you describe KSHV and its association with HIV/AIDS?

KSHV is endemic in the areas of Africa afflicted by the HIV/AIDS epidemic and leads to what is known as an HIV-associated cancer, Kaposi’s sarcoma. This condition develops in patients with compromised immune systems and so, historically, has been associated primarily with the elderly, and more recently with organ transplant recipients. However, the immunosuppressive nature of HIV/AIDS has led to a massive increase in cases of this cancer in Africa. Importantly, while Kaposi’s sarcoma is seen as a third-world disease – HIV treatment in the Western world prevents the immunosuppression required for its development – it has increased to become the most common form of cancer in men in sub-Saharan Africa and so poses a massive public health problem for large parts of the world.

Your postdoctoral investigations centred on the early lytic protein of KSHV termed ORF57. Why did you decide to focus your efforts on this particular target?

KSHV is unique amongst the oncogenic herpesvirus in that it requires both the latent and lytic phase of the life cycle for progression of Kaposi’s sarcoma (only latent infection is required for other oncogenic herpesviruses). Therefore, studying the proteins that regulate the switch between latency and lytic replication could allow us to block lytic replication and therefore prevent the onset of cancer. ORF57 is one of the key proteins in controlling this switch, and is responsible for exporting all the viral mRNAs from the nucleus into the cytoplasm.

Can you discuss the necessity to understand the mutations in the proteins involved in RNA export from a cell’s nucleus to the cytoplasm? How do mutations in these RNA export proteins lead to DNA damage?

Only very recently have we begun to understand the importance of mutations in mRNA export proteins. Exporting mRNAs from the nucleus to the cytoplasm is essential for translation and so aberration in export proteins can lead to altered expression patterns of multiple proteins that cause disease. Moreover, the processes of transcription, splicing, export and translation are intimately linked, and mRNA export proteins may have additional roles throughout the life of an mRNA. A novel mechanism of DNA damage caused by mutations in RNA export proteins is termed RNA-mediated instability. Their loss of function can lead to a decrease in the stability of newly transcribed mRNA, causing RNA:DNA hybrids to form between the newly transcribed RNA and the template strand of the DNA – termed an R-loop. These hybrids are now known to be causes of DNA damage in cancer, most recently linked to BRCA2-associated breast cancer.

How significant has your collaboration with Professor Adrian Whitehouse at the University of Leeds been to your investigations? Will you be cooperating on any future research efforts?

The collaboration with Professor Whitehouse has been essential for my recent studies. The preliminary data that led to my current position were attained while working as a postdoctoral researcher in his laboratory. Since then, he has continued as my academic mentor throughout my fellowship, and discussions and sharing of consumables have allowed me to progress with the investigations as well as I have. I aim to move away from herpesvirus biology in my future research, but the strong links with the Whitehouse group, and particularly with their expertise in mRNA export, mean that we intend to collaborate closely on future research endeavours.

Dr Brian Jackson discusses how his extensive background in DNA repair mechanisms has led to his current research on RNA processing defects in human cancer formation and progression.
Replication regulation

At the University of Leeds’ School of Molecular and Cellular Biology, breakthrough discoveries in oncogenic herpesvirus research is leading to highly promising targets for the development of novel anticancer therapeutics.

**FIRST DISCOVERED IN** 1994, Kaposi’s sarcoma-associated herpesvirus (KSHV) is the eighth member of the Herpesviridae family. In the higher income countries of the Western world rates of KSHV infection are low and do not commonly present any symptoms for the otherwise healthy individual. For the immunosuppressed, however, KSHV presents a very real danger; unlike some of its other ubiquitous Herpesviridae family relatives, infection with KSHV in affected individuals may lead to primary effusion lymphoma, multicentric Castleman’s disease or Kaposi’s sarcoma, an aggressive tumour originating in the lymphatic endothelium.

Considered to be a rare cancer in the West, in sub-Saharan Africa Kaposi’s sarcoma poses a serious health risk with rates reaching the scale of an epidemic that mostly affects those with weakened immune systems, such as people with AIDS or organ transplant recipients. While the precise route of KSHV transmission remains uncertain, efforts to quell the epidemic are trying to identify novel targets in order to develop effective therapeutics. A solution may be found in KSHV’s unique infection strategy, which makes use of both latency and lytic replication. Since lytic replication drives the spread of infection, pathogenesis and tumorigenicity, a better understanding of this critical phase could be key to combating this epidemic.

At the University of Leeds’ School of Molecular and Cellular Biology, Dr Brian Jackson’s research on KSHV aims to reveal the molecular mechanisms that enable lytic replication to take place and the implications of RNA processing in cancer development. Having specialised in DNA repair mechanisms whilst at the University of East Anglia, Jackson followed his interests in nucleic acid processing with a postdoctoral position at Leeds alongside Professor Adrian Whitehouse. Their investigations into KSHV infection have unearthed a surprising relationship between lytic replication and DNA damage.

Awarded the Wellcome Trust’s Institutional Strategic Support Fund Junior Development Fellowship to explore the relationship further, Jackson’s independent research continues to benefit from a strong collaboration with the Whitehouse group.

**VIRAL/CELLULAR INTERACTIONS**

Lytic replication has been identified as a phase necessary for Kaposi’s sarcoma to develop from its initial target site, the B lymphocyte reservoir, to the endothelial cells where tumourigenesis occurs. In the production line of gene expression, nuclear transcription is followed by a series of RNA processing events, including the mRNA’s translation into proteins. In order to do this, the human transcription/export complex (hTREX) needs to transport the transcribed mRNA from the host cell’s nucleus to its cytoplasm. This mRNA export is intimately linked to cellular splicing. The majority of KSHV mRNAs, however, have no introns. What, then, are the molecular mechanisms regulating lytic replication and ensuring the completion of mRNA biogenesis in this oncogenic herpesvirus?

Jackson’s research has focused on a multifunctional viral mRNA export factor named open reading frame 57 (ORF57), which exists as a functionally conserved homologue in all human herpesviruses. In their collaborative work Jackson and Whitehouse have found evidence to suggest KSHV has evolved in a way that allows ORF57, despite being primarily a transport and accumulation protein, to affect every stage of mRNA biogenesis. By interacting with multiple cellular proteins, this viral export factor is able to recruit hTREX in its entirety to the lytic viral mRNA so that it can be transported from the nucleus to the cytoplasm, despite having no introns, ready for translation of the viral proteins.

Using biochemical analyses and functional assays, the cellular protein PYM has recently been identified as playing a pivotal role in the successful translation of viral mRNAs. Having
observed that it binds to intronless KSHV mRNA via an interaction with ORF57. Jackson has also demonstrated that when PYM is depleted, the interactions between ORF57 and the host cell’s translational machinery significantly drops. Indeed, by decreasing functional PYM a specific decrease in the efficiency of translation can be observed, demonstrating ORF57’s ability to enhance this process through the recruitment of PYM.

Jackson and Whitehouse’s investigations have also highlighted the importance of two other major components of the hTREX complex, the cellular export proteins Aly and UAP56 interacting factor (UIF), in the latter case demonstrating the first known interaction between UIF and a viral protein. ORF57’s relationship with these proteins is necessary for efficient export of KSHV mRNA but does not require both, highlighting a redundancy in the eukaryotic system. Interacting with either UIF or Aly alone, ORF57 can carry the mRNA to the cytoplasm, but without either, the whole process is halted in its tracks. Complemented by the identification of the residues necessary for interaction between an ORF57 homologue and Aly, these structural insights into the mechanisms regulating KSHV’s lytic replication cycle mark an early but important step toward the development of potential therapeutics.

**DAMAGED DNA**

Primarily an mRNA export protein, the effects of ORF57 expression on cells is not expected to have much of an impact on DNA. Large scale proteomics screenings, however, have unearthed surprising results: when ORF57 recruits hTREX to the viral DNA, RNA-DNA structures known as R-loops are formed at the point of cellular transcription. In essence, these R-loops mimic mutations in the hTREX complex and damage the DNA. “DNA damage, or genome instability, is known as an enabling characteristic of the six hallmarks of cancer,” explains Jackson. Now understood to directly contribute to the driver mutations required for Kaposi’s sarcoma progression, the impact of these findings are expected to help reveal the role of hTREX mutations in the progression of a variety of other cancers.

As yet, this mechanism of RNA-mediated genome instability is still a relatively recent discovery and remains to be fully elucidated. To this end, Jackson’s endeavours are beginning to include the wider context of human cancers, focusing on the effects of RNA processing defects on tumourigenesis in order to reveal the exact mechanism by which mutations in RNA export proteins induce DNA damage. It is these kind of studies that will help improve the potency of current anticancer therapies by producing a detailed knowledge of potential targets. In KSHV, it is now known that ORF57 may contribute to Kaposi’s sarcoma development, as shown by the virus’ inability to produce progeny virions when ORF57 has been removed. Likewise, Jackson and Whitehouse’s investigations into the interactions between viral and cellular proteins demonstrate highly promising targets for potential therapeutic development. Similar to the wholesale removal of ORF57, strategies designed to block its pairing with PYM, Aly or UIF have repeatedly shown that KSHV’s ability to generate progeny virions can be dramatically impaired.

Having come far in painting a clearer picture of the molecular mechanisms regulating the lytic replication cycle, just how little it is fully understood is emphasised by the recent discovery of yet more hTREX components. Although further surprises may be in store, it also adds to the continuing improvement of anticancer treatments. Now, by blocking the interactions of ORF57, it will be possible to halt KSHV’s lytic replication and stop the progression of Kaposi’s sarcoma.