

IRES advances and applications

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX - 23 October 2007

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chair:** *Dr Lisa Roberts*, University of Surrey, UK
- 10:00 – 10:30 **Picornavirus IRES elements; variations on a theme**
Dr Graham Belsham, National Veterinary Institute, DTU, Denmark
 IRES elements were first characterized within the RNAs from various picornaviruses including poliovirus, human rhinoviruses, encephalomyocarditis virus and foot-and-mouth disease virus. These represent examples of two different types of IRES element. However, it is now known that there are 4 different classes of picornavirus IRES element, these differ in size, secondary (and presumably tertiary) structure and their requirement for cellular translation initiation factors plus other trans-acting factors. Remarkably, one of these types of picornavirus IRES element is very similar to the IRES elements found within hepatitis C virus and other members of the flaviviridae (e.g. the pestiviruses).
- 10:30 – 11:00 **The role of RNA structure in picornavirus IRES activity**
Encarna Martinez Salas, Centro de Biología Molecular, Madrid, Spain
 Understanding of internal ribosome entry site (IRES) function requires a detailed knowledge of each step involved in the internal initiation process, from RNA folding and IRES-protein interaction to ribosome recruitment. Thus, deciphering the IRES accessibility to external agents due to RNA structural features as well as RNA-protein protections within living cells is of primary importance. The accessibility of unpaired nucleotides in the entire foot-and-mouth disease (FMDV) IRES has been obtained *in vitro* by RNA probing techniques; subsequently it has been used to interpret the footprint data obtained *in vivo* for the mRNA encompassing the IRES element in the intercistronic space. Two chemical reagents, dimethyl-sulfate and AMT-psoralen, that enters the cell membrane and interact with nucleic acids in a structure-dependent manner have been used to footprint the FMDV IRES in living cells in the context of a biologically active mRNA. The results of DMS accessibility and UV-psoralen crosslinking in the competitive cellular environment evidenced differences in RNA structure with data obtained *in vitro*.
- 11:00- 11:10 **Group and speakers photo**
- 11:10 – 11:30 **Mid-morning break**
- 11:30 – 12:00 **Structural and functional analysis of IRES trans-acting factors**
Dr Stephen Curry, Imperial College, UK
 The IRES elements of picornaviruses display a requirement for a diverse array of host-cell RNA binding proteins, known as IRES trans-acting factors (ITAFs), many of which are not normally involved in translation initiation. The mechanism of action of these ITAFs is poorly understood, largely because structural studies of IRES-protein complexes present stiff challenges to investigators. Our group has focused on elucidating the solution and crystal structures of a number of distinct ITAFs, including PTB, La and ITAF45. These studies have provided many valuable insights and are laying important groundwork for future investigations. Recent highlights will be summarised.
- 12:00 – 12:30 **Trans-acting factors of cellular and artificial IRESes**
Dr Keith Spriggs, Nottingham University, UK
- 12:30 – 12:50 **Tour of the BioPark**

12:50 – 14:00 **Lunch and Poster Viewing**

14:00 – 14:30 **Effect of oxidative stress on HCV IRES-mediated and host cell translation.**

Dr Shiu-WanChan, University of Manchester, UK

Hepatitis C patients very often present elevated levels of oxidants in the blood and liver. Thus, the potential of anti-oxidants in anti-HCV therapy has been recognized and compounds of this type are now in clinical trials. Before we can fully appreciate the potential of anti-oxidants in anti-HCV therapy we must understand the effects of oxidative stress on the virus. HCV utilises an IRES element for translation, in contrast to cap-dependent translation of the majority of cellular proteins. To understand how virus translation is modulated under oxidative stress, we compared HCV IRES-mediated translation with cap-dependent translation using a bicistronic reporter construct.

14:30 – 15:00 **IRES-mediated translational regulation of FGF-1 expression during myogenesis and muscle regeneration**

Dr Caroline Conte, Institut National de la santé et de la recherche médicale, France

FGF-1 is expressed as a single protein, under the control of four distinct promoters allowing synthesis of four transcripts. We identified IRESs in transcripts A, B and C. We studied the regulation of FGF-1 expression in differentiating myoblasts and in a mouse model of muscular regeneration. Promoter A and the IRES A are activated concomitantly with expression of endogenous FGF-1. These results reveal a unique co-regulation of promoter and IRES, suggesting a mechanism of coupled translation and transcription. In addition, IRES A is an excellent candidate to be used in multicistronic vectors aimed to co-express therapeutic molecules against myopathies

15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**

15:30 – 16:00 **Structural and biochemical studies of the HCV IRES**

Dr. Peter Lukavsky, MRC LMB, UK

IRES RNAs from the hepatitis C virus and classical swine fever virus coordinate cap-independent assembly of eukaryotic 48S initiation complexes. Here we report that these IRESes also play a functional role during 80S ribosome assembly downstream of 48S complex formation, in promoting eIF5-induced GTP hydrolysis and eIF2/GDP release from the initiation complex. We show that this function is encoded in their independently folded IRES domain II and that it depends both on its characteristic bent conformation and conserved RNA motifs. Our data suggest a general mode of subunit joining in HCV and HCV-like IRESes

16:00 – 16:30 **Insect virus IRES elements-functional analysis and utility in protein expression systems**

Dr Lisa Roberts, University of Surrey, UK

We have previously demonstrated that the 5' untranslated region of the *Rhopalosiphum padi* virus (RhPV) genome contains a novel internal ribosome entry site (IRES) element that functions in mammalian, insect and plant *in vitro* translation systems. We have recently defined the minimal sequences required for directing internal initiation in mammalian (RRL), plant (WGE) and insect (Sf21 cells) translation systems and have shown that the 3' unstructured region within the 5'UTR seems to be critical for IRES function. Use of this IRES element in the generation of an improved baculovirus expression vector will also be described.

16:30 – 17:00 **Chairman's summing up & close.**