Ferenc Muller, University of Birmingham
Functional validation of disease associated human enhancer candidates using the zebrafish transgenic embryo as a scalable vertebrate model

Genome wide analyses such as ENCODE and FANTOM predicted a surprisingly large number of non- coding elements with suggested cis-regulatory function in mammals. However, functional validations of the candidate regulatory elements are mostly lacking due to limitations of transgenesis technologies and capacities. We have investigated the utility of the transgenic zebrafish embryo as a scalable in vivo vertebrate model to study the functionality of candidate human enhancers predicted by a combination of chromatin signatures (e.g. H3k4me1, H3K27 Ac, H2AZ), TF binding events and enhancer specific bidirectional transcription. These analyses indicated that despite the evolutionary distance between human and fish, 60% of the candidate enhancers exhibiting sequence conservation lead to reporter expression which recapitulates the expression patterns of either zebrafish or human genes associated with the candidate enhancers. For example, we have demonstrated the activity of enhancer candidates associated with Type 2 diabetes and fasting glycemia in the pancreatic islet of zebrafish larvae. Currently we are developing assays in order to improve the sensitivity of transgenesis tools so as to detect subtle changes in enhancer function caused by sequence variation (SNPs) associated with dysregulation in disease. To improve the reliability of zebrafish enhancer function assays, a targeted integration system mediated by PhiC31 integrase was developed with which we have demonstrated successful elimination of position effect variation commonly found in conventional, transposon-based transgenesis.

Tibor Pankotai, University of Szeged
Mechanistic insights into the transcriptional arrest in the presence of Double Strand Breaks

Double-strand breaks (DSBs) occur frequently in the genome during genome replication or by DNA damaging agents. DNA lesions affect fundamental DNA-dependent nuclear processes such as replication and transcription. We have developed an experimental system where DSBs are induced at coding regions of RNA polymerase II transcribing genes. We have started to study the kinetics of RNA polymerase II transcription inhibition in the presence of DNA breaks. We observed that induction of the break led to transcription inhibition and the restoration of transcription closely followed the dynamics of the repair of breaks. We confirmed by chromatin-immunoprecipitation that the break induction led to displacement of RNA polymerase II affecting both the elongation and the initiation of transcription. Our results show that this is dependent on one of the major kinase in DNA damage repair called DNAPKcs. We also investigated the downstream steps of RNA polymerase II removal and we claimed that it was a multistep process involving additional kinases and ubiquitin ligases NEDD4 and CUL3. At the last step of break dependent transcriptional silencing the RNA polymerase II is targeted for proteasome dependent degradation. These data demonstrate that the DNA damage repair complexes and proteasomal system have a synergistic and active role in transcriptional silencing during the DSB repair by removing the RNA pol II from the transcribing region. We show here that DNA lesions occurring at transcribed regions cause a transient repression until the lesion is repaired. This is probably a cell defense mechanism to avoid production of truncated or mutated transcripts in essential genes whose alterations in their gene expression would endanger cell viability. Understudying the role of DNAPKcs, in preventing RNA pol II bypassing a DSB might be a key in avoiding the production of mutated transcripts that could lead to cancerous phenotypes.

18.35-18.45 **Demonstration of world's fastest qPCR system xxpress (40 PCR cycles in 10 minutes)**

18.45- 20.00 Wine and Cheese and Posters