BORDEAUX RNA CLUB SEMINAR

Thursday, September 29th 2011

At the European Institute of Chemistry and Biology

Programme

11.00 – 12.00 *RNA invention from eukaryotes to archae: a combined bioinformatics & RNA-seq view*

Dr. Daniel Gautheret, Institut de Génétique et Microbiologie - UMR CNRS 8621, Université Paris-Sud

Abstract: Les ARN non-codants peuvent apparaître par duplication, exaptation d'anciens gènes ou «invention» pure et simple à partir de transcrits non fonctionnels. A partir d'analyses phylogénétiques et de séquençage massifs d'ARN réalisés au laboratoire sur des organismes très variés (la bactérie Vibrio splendidus, le riz, une archée hyperthermophile), je montrerai la très grande fluidité du transcriptome non-codant dans tous les domaines du vivant.

12.00 – 13.00 **Lunch (mandatory registration at u869@inserm.fr)**

13.00 *An essential role for Clp1 in the architecture of the polyadenylation complex CF IA*

Dr. Lionel Minvielle-Sébastia, Directeur de Recherche CNRS, Inserm U869/IECB

Abstract: Polyadenylation is a cotranscriptional process that modifies mRNA 3' ends in eukaryotes. In yeast, CF IA and CPF constitute the core 3'-end maturation complex. CF IA comprises Rna14p, Rna15p, Pcf11p and Clp1p. CF IA interacts with the C-terminal domain of RNA Pol II largest subunit via Pcf11p which links pre-mRNA 3'-end processing to transcription termination. We analyzed the role of Clp1p in 3' processing. Clp1p binds ATP and interacts in CF IA with Pcf11p only. Clp1 mutants abolishes transcription termination at snoRNA genes. Moreover, we found that association of mutations in the ATP-binding domain and in the distant Pcf11p-binding region impair 3'-end processing. Strikingly, these mutations destroy CF IA architecture. ChIP experiments showed that Rna15p crosslinking to the 3' end of a protein-coding gene is perturbed by these mutations whereas Pcf11p is only partially affected. This study reveals an essential role of Clp1p in CF IA organization. We postulate that Clp1p transmits conformational changes to RNA Pol II through Pcf11p to couple transcription termination and 3'-end processing. These rearrangements likely rely on the correct orientation of ATP within Clp1p.

13.30 *Analyzing miRNA biology: workflow and tools to identify and validate miRNA gene targets*

Dr. Stéphanie Urschel, Senior Field Scientist, Thermo Fischer Genomics

Abstract: Although microRNAs (miRNAs) have been shown to play roles in important biological processes such as development, differentiation and disease, characterizing their gene targets remains a challenge. The miR-200 family has been shown to regulate epithelial to mesenchymal transitions (EMT), a process that is critical for normal development and tumor metastasis. Furthermore, recent publications indicate that the miR-200 family regulates EMT by targeting ZEB1 and ZEB2, transcription factors that repress E-cadherin. While targeting of ZEB1 and ZEB2 by the miR-200 family appears to be important for maintaining an epithelial phenotype, additional gene targets of the miR-200 family may contribute to regulating EMT. Here we describe the workflow and tools applied to identify and validate additional miRNA gene targets. Using MCF7 and MDA-MB-231 cell lines as models for EMT and MET, cells were transfected with miRNA inhibitors and mimics, respectively. RNA isolated from these transfected cells was analyzed for changes in mRNA expression using whole genome microarrays and microfluidic high-throughput qPCR. Potential targets regulated by the miR-200 family were identified by focusing on a collection of genes that are inversely regulated by mimics and inhibitors; this list of potential targets included novel gene targets that are not predicted by standard algorithms. Putative gene targets were validated by demonstrating miRNA targeting of the gene's 3'UTR and correlating phenotypic effects of target gene silencing with miRNA effects.

14.00 *Studying small non-coding RNAs in the bacteria Helicobacter pylori*

Hélène Arnion, Equipe sRNA PAR, Inserm U869, Bordeaux

Abstract: Helicobacter pylori is a bacterial pathogen that naturally colonizes the human gastric mucosa of more than half of the world's population. This infection can lead to peptic ulcer diseases, gastritis and gastric cancers. During the last few years, bacterial small regulatory RNAs appeared as a new class of key regulators in genes expression. They usually target mRNAs, bind to them by base pairing, and then, act as inhibitors or activators of mRNA translation. They regulate many different genes, encoding e.g., virulence factors, membranes proteins or endogenous toxins. By using combined bioinformatic and experimental (deep sequencing) approaches, we discovered a novel family of small non coding RNAs in the sequenced 26695 H. pylori strain. This family is composed of six homologous genes (IsoA1 to 6), located at different loci in the genome. We also identified a new class of mRNAs, expressed from the opposite strand of the asRNAs. Each of these new mRNAs encode a conserved short peptide (30 amino-acids) and thus are named AapA. This mRNA and its small cis-encoded antisense transcript (IsoA) are both expressed in vivo, defining a small expression cassette, repeated many times in the genome of different strains. We have shown that the peptides encoded by the AapA are translated in vitro. They are positively charged and may fold as a-helices with distinct hydrophobic and charged surfaces, making them good candidates for being toxic in H. pylori. Surprisingly each asRNA is able to repress peptide translation in vitro, with a high affinity and specificity for its own cis-encoded mRNA.