BORDEAUX RNA Club

Thursday December 12th, 2013

At the European Institute of Chemistry and Biology

14.00–15.00 ***Trm112, a unique methyltransferase activator at the interface between ribosome synthesis and function***

Dr. Marc Graille, Ecole Polytechnique, Paris, France

Abstract: Trm112, a small zinc finger protein of 15 kDa [1,2], is known to interact with, and to activate, four methyltransferases (MTases), namely: Mtq2, Trm9, Trm11 and Bud23, all related to ribosome function. The Mtq2-Trm112 complex methylates the translation termination factor eRF1 on the glutamine side chain of its universally conserved GGQ motif, which is directly involved in the release of newly synthesized proteins [1,3]. The Bud23-Trm112 complex is implicated in ribosome biogenesis by methylating guanosine 1575 of 18S rRNA and the gene encoding for Bud23 human orthologue is deleted in patients suffering from Williams syndrome, a multisystem developmental disorder [4]. Trm11-Trm112 forms 2-methylguanosine at position 10 on tRNAs, a modification assumed to stabilize tRNA structure. The Trm9-Trm112 complex participates in the modification of wobble uridine 34 of some tRNAs. It catalyses the methylation of the cm5U (5-carboxymethyl Uridine) into mcm5U (5-methoxycarbonylmethyl Uridine) which is the final product of the modification. This methylation enhances the decoding accuracy of specific codons highly represented in some key genes of DNA damage response. ALKBH8, the human orthologue of Trm9 is over-expressed in various types of cancer, including bladder cancer. Moreover, the human gene is located in a chromosomal region, which is often deleted in colorectal tumours. Trm112 is truly unique acting as an activating platform of four MTases involved in rRNA, tRNA and translation factor modifications, ideally placing it at the interface between ribosome synthesis and function. The structural and functional studies conducted on these Trm112-MTase complexes will be presented.

(1) Heurgué-Hamard et al (2006) J. Biol. Chem.
(2) Graille et al (2012) Biochimie.
(3) Liger et al (2011) Nucleic Acids Res.
(4) Figaro et al (2012) Mol. Cell. Biol.

15.00–15.30 ***A proton wire to couple substrate accommodation and peptide bond formation on the ribosome***

Dr. Axel Innis, Institut Européen de Chimie et Biologie, Bordeaux, France

Abstract: Peptide bond formation takes place on the large subunit of the ribosome within an active site that is mainly composed of RNA. During this process, the terminal amine of an aminoacyl-tRNA attacks the ester carbonyl carbon of a peptidyl-tRNA to yield a deacylated tRNA and a peptidyl-tRNA that is lengthened by one amino acid.The estimated 10^7-fold rate enhancement relative to the uncatalyzed reaction has been largely attributed to a decrease in the entropy of activation. However, both the roles of specific residues in the reaction and the strategy used to deprotonate the attacking nucleophile remain unclear. Using X-ray crystallographic analysis to study pre-attack and post-catalysis 70S ribosome complexes with tRNAs at 2.55-2.7 Å resolution, we show how a previously overlooked network of hydrogen bonds could ensure that the nucleophilic attack and deprotonation are coordinated in the rate-limiting step to yield a tetrahedral intermediate. Moreover, we suggest a supporting role for ribosomal protein L27 during catalysis, thus illustrating how the catalytic activity of a prebiotic ribozyme may have been enhanced through the recruitment of a protein.

15.30–16.00 ***Overexpression of miR-10a in atypical myeloproliferative disorders: causes and consequences***

Dr. Eric Lippert, CHU de Bordeaux, France

Abstract: Myeloproliferative disorders result from acquired genetic anomalies in hematopoietic stem cells responsible for an excessive proliferation in the myeloid compartment. For example, in Chronic Myelogenous Leukemias (CML), a fusion between the BCR and ABL1 gene produces a chimeric, consitutively activated tyrosine-kinase, BCR-ABL1 that stimulates the growth of the myeloid cells. Atypical CML is a very rare disorder that closely ressembles CML, but also displays elements of myelodysplasia (anomalies in the differentiation programs). Its pathogenesis is not as clear as this of CML and no constant genetic abnormality is found. Since micro-RNAs have been shown to participate in the pathogenesis of certain leukemias, we studied the «miRNome» of peripheral blood cells from patients carrying CML, aCML or reactive hyperleukocotosis as controls. One of the most deregulated miRNAs, overexpressed in aCML patients' cells was miR-10a, a micro-RNA encoded by the HOXB gene locus and the expression of which is altered in various cancers. HOXB genes are important for hematopoiesis and are regulated by epigenetic mechanisms. Epigenetic modifying drugs are commonly used in the treatment of myelodysplasias and recently, genes encoding epigenetic modifying enzymes were found mutated in a number of myeloproliferative/myelodysplastic neoplasms. For these reasons, we looked whether miR-10a expression was modified by epigenetic targeting drugs. We showed that optimal expression of miR-10a (and HOXB4) required DNA demethylation and histone deacetylation. However the level of expression of miR-10a in patient cells did not correlate with the mutational status of DNMT3A, EZH2, ASXL1, TET2 or IDH1/2. MiR-10a has been found overexpressed in various hematological malignancies including Acute Myeloblastic Leukemias, but its function in hematopoiesis is unclear. We modified miR-10a expression in hematopoietic cell lines and in primary hematopoietic progenitor/stem cells to study its role in proliferation and differentiation. In the various models, no modification was found in proliferation or differentiation of the most mature cells. However, stem cell self renewal seems altered by miR-10a expression. In conclusion, miR-10a is overexpressed in some patients presenting aCML and other myeloid malignancies. The reasons for this deregulation may include epigenetic alterations, but do not seem clearly correlated with the mutational status of epigenetic genes. The role of miR-10a on stem cell biology may explain its function in leukemogenesis.

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