

Thesis Title	Ribonucleotides in DNA and consequences on genome homeostasis: a case of type 2 RNase H in <i>Archaea</i>
Contract type	Contract to support training through research
Department/Office	Laboratory of Microbiology of Extreme Environments (LMEE), PDG/REM/BEEP/LMEE.
Duty station	Ifremer Brittany Centre, Plouzané (France)
Date of issue :	
Doctoral School Thesis supervisor Co-supervisors	Marine Science PhD School, Plouzané (France) Ghislaine Henneke
Reference (HRD)	

The Institute and the recruiting department

The laboratory (LMEE UMR BEEP Ifremer, CNRS UBO) is interested in the microbiology of deep marine extreme environments through the study of ecosystems, the description of biodiversity, and the understanding of adaptive processes at the cellular and molecular levels; and in particular in the genomic maintenance at high temperature in hyperthermophilic *Archaea* including ensemble and single-molecule biochemical approaches, genetics, proteomics and structural biology. <https://www.umr-beep.fr/>

Summary

RNA-containing structures can form within genomic DNA. Ranging from ribonucleotide (rNMPs) insertions to RNA-DNA hybrids, they represent potential obstacles to DNA transactions. Compatible with genome homeostasis, cells evolved dedicated DNA repair and tolerance mechanisms. Among them, Type 2 RNase H mediated pathway is evolutionary conserved in all domains of life. However, many questions remain about the molecular mechanism and regulation of RNase HII/2's intertwined DNA replication and DNA repair activities. In this PhD project, it is intended to decode the role of crucial amino acids which governs substrate specificities of RNase HII in Thermococcales. Based on biochemical and X-ray structural preliminary data, RNase HII mutants will be generated to further elucidate the binding and kinetics towards embedded rNMPs and RNA-DNA hybrids *in vitro*. They will also be screened for protein crystallisation followed by structural investigations with our collaborator in Japan (D^r R. Uehara). These active site mutants will be genetically constructed and their phenotypic variations analysed. The occurrence of ribonucleotides in DNA and their location at the genome-wide scale will be carried out in wild-type and mutant Thermococcales cell lines. Similarly, the binding sites of RNase HII on the genome of wild-type and mutant cell lines. will be characterized.

Key words

Embedded rNMPs ; RNA-DNA hybrids ; Type 2 RNase H ; Genome stability ; Deep Sea Thermococcales ; single-molecule TIRF microscopy



Expected profil

The student should have a postgraduate training in Biochemistry / Molecular Biology / Microbiology. Laboratory experience is crucial in procedures such as cloning, bacterial cultivation, production and purification of proteins and enzyme assays. The chosen applicant should be motivated for biochemical research and functional genomics. She (he) should have good communication skills, scientific writing and data analysis. She (he) will be working in a collaborative and international environment.

Specific working conditions

The PhD project will be carried out at the Laboratory of Microbiology of Extreme Environments, Ifremer Plouzané. The PhD student might benefit, under certain conditions, from a short-term international mobility in the laboratory of Dr R. Uehara in Japan.

Phd is a real opportunity to work on Ifremer's scientific and technological priority themes. It entitles the holder to a gross monthly salary of 2300 euros gross for a period of 3 years, which cannot be combined with other scholarships.