PhD project available in Toulouse, France

Project title: Gene regulation via RNase Y cleavage-specificity in the human pathogen *Staphylococcus aureus*

Project start: 1st of October 2017
Duration: 3 years
Funded by: Axes Thématiques Prioritaires de l'Université Paul Sabatier

Research team: RNA decay and gene regulation in *Staphylococcus aureus*
Directed by Peter Redder
LMGM, Centre de Biologie Integrative
Paul Sabatier University, Toulouse, France
Contact for further information: peter.redder@univ-tlse3.fr or (+33)561335981
Webpage of the team: https://www-lmgm.biotoul.fr/uk/equipes/grpredder/

Overview

RNA degradation is a major transcriptome-wide regulatory mechanism. The proposed project will examine the biology behind the specificity of many of the degradation RNases (with focus on RNase Y), a specificity which is, for example, needed for the tight control that *Staphylococcus aureus* (a model organism for Gram-positive pathogens) keep on its virulence factors.
A key point of the project is to explore RNA decay on a transcriptome-wide scale, using Next-Generation Sequencing protocols that have been specifically developed for this purpose by the Redder lab, and to use the data generated to obtain an integrative view of the gene regulation systems of *S. aureus*. Bioinformatic analyses will provide insight into RNA cleavage, maturation and degradation patterns on a large-scale, but will also allow the student to scale down to individual events, which can (and will) be examined in molecular detail.

Candidate qualifications

Candidate must have obtained a master (or equivalent) degree by 1st of October 2017.
The successful candidate should be interested and knowledgeable in molecular biology and/or biochemistry, and previous experience in one or both of these fields is a large plus.
Language: English is required, but French is not essential (although a willingness to learn is a plus).

Project description

One of the key phases of regulating gene expression in all cells, is to ensure the correct level of mRNA and thus the correct potential for protein synthesis. If an RNA species is completely repressed under normal circumstances, then an activation or de-repression of its promoter can quickly increase its intracellular concentration. However, if down-regulation of an already abundant RNA is needed, then regulation at the synthesis level is futile unless RNA half-lives are kept sufficiently short to allow a rapid removal of existing RNA molecules. Indeed, most of the mRNAs in bacteria have short half-lives ranging from seconds to a few minutes, and the pool of a given RNA is thus in a constant flux, where its level is determined by a combination of its synthesis rate and its decay rate. This highlights a central paradigm of this project, namely **that RNA degradation must be highly efficient, but should at the same time be limited, to avoid killing the cell by elimination of all cellular RNA**.
Staphylococcus aureus is model organism for the many Gram-positive pathogens, and is an ongoing health-problem in both the Western and developing world. *S. aureus* is an opportunistic pathogen, which is part of the normal nasal microbiota in about 30% of the human population. Many cellular processes, such as the switch from peaceful coloniser to

aggressive pathogen, requires precise and specific regulation. This is, at least in part, achieved my having precise and specific initiation of RNA decay.
Three of the key RNases in *S. aureus* (**RNase J1, J2 and RNase Y)** have no homologs in *E. coli*, but are widespread elsewhere in the bacterial domain. RNase Y is attached to the inside of the membrane, and cleaves at least 100 targets with high specificity and reproducible cleavage sites. Consequently, **the focus of this PhD project will be to uncover the molecular interactions and requirements that govern the specificity of RNase Y**.
A small number of cases are known where adjacent RNA elements are important for RNase Y target selection, and one of the first tasks in the project will be to mutate these sequences to identify what "attracts" RNase Y. This will then be followed up by transcriptome-wide analyses to integrate a large number of cleavage sites into a model.
In parallel the student will study the protein interactions and localisation of RNase Y, which, due to its membrane localisation, can only cleave RNAs that are near the membrane. Deletion of the RNase Y membrane anchor leads to poor growth, probably due to loss of specificity. Are the RNAs transported (or diffused) to the membrane? Is a specific protein/complex responsible for this? Which RNAs are selected? (and how?)

Experimental and analytical approaches
Next-Generation Sequencing methods to examine RNase Y cleavage in a large scale.
Bioinformatics to analyse the transcriptomic sequencing data, and to determine the factors that govern cleavage specificity.
Molecular biology for construction of altered cleavage sites, to verify to dismiss hypotheses.
Genetic and Microbiological screens and selection for mutants that modify RNase Y activity.
Biochemical assays to test specificity in isolated *in vitro* models, with and without co-factors.

About the research team
The "RNA decay and gene regulation in *Staphylococcus aureus*" research team is newly established in the Centre de Biologie Integrative, at the campus of University of Toulouse III.
Prof. Peter Redder was previously in Geneva (Switzerland), where RNA decay and gene regulation in *Staphylococcus aureus* was studied since 2012. The team currently consists of Fatouma Houssein, Gladys Munoz, Celine Pelissier, and Peter Redder.

Litterature

Kirkpatrick CL, Martins D, Redder P, Frandi A, Mignolet J, Chapalay JB, Chambon M, Turcatti, G, Viollier PH. Growth control switch by a DNA-damage-inducible toxin–antitoxin system in *Caulobacter crescentus*. Nature Microbiology, 2016, 16008. doi:10.1038/nmicrobiol.2016.

Khemici V, Prados J, Linder P, Redder P. Decay-initiating endoribonucleolytic cleavage by RNase Y is kept under tight control via sequence preference and sub-cellular localisation. PLoS Genet. 2015 Oct 16;11(10):e1005577.

Linder P, Lemeille S, Redder P. Transcriptome-wide analyses of 5'-ends in RNase J mutants of a Gram-positive pathogen reveal a role in RNA maturation, regulation and degradation. PLOS Genetics. 2014 Feb 27;10(2):e1004207. doi: 10.1371/journal.pgen.1004207.

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Redder P. How Does Sub-cellular Localization Affect the Fate of Bacterial mRNA? Curr Genet. 2016 Mar 14.