Invasion and proliferation of the bacterial agent of listeriosis Listeria monocytogenes in human cells trigger drastic changes in gene expression and cell function; typically host cells launch antibacterial defences while pathogens subvert various host pathways to their own benefit. Our group Bacterial Infection and RNA Destiny aims to explore the consequences of the infection on eukaryotic mRNA stability and translation, to unravel its molecular mechanisms, and to highlight its physio-pathological consequences.

We have substantial evidence that during the infection of epithelial cells by Listeria monocytogenes (Lm), the food-borne cause of listeriosis, host cell gene expression is reshaped quantitatively and qualitatively by a combination of regulations affecting RNA synthesis, maturation, decay and translation. We have recently gathered different sets of -omics data covering with high resolution the flux of host gene expression from transcription to translation during an infection time-course (0, 2, 5, 10 h) in intestinal human epithelial cells using the following technologies: RNA-Seq, 4xU-Seq of nascent RNA, Ribo-Seq of translatome, and Nanopore MinION full-length cDNA sequencing. Our initial statistical analysis of these datasets, experiment by experiment, has confirmed their quality and reliability, and further revealed groups of transcripts that are induced or repressed during infection, transcriptionally, and/or translationally (Besic et al., in preparation). Among the regulated transcripts, some impact the outcome of infection. These not only include transcripts encoding proteins involved in innate immune responses, but also several actors of host translational control, such as the translational regulator [fill in]. This regulator of host translation has been previously associated with inflammatory responses, but precise understanding of its role during viral or bacterial infections is clearly missing.

Preliminary analysis of our data has also revealed variations in RNA processing events, such as delays in splicing, splicing isoform switching, or lack of precision in transcription termination. The analysis and interpretation of full-length cDNA sequencing (MinION) is in progress; it has already uncovered that differential splicing of the [fill in] pre-mRNA leads to the use of a distal 3’-UTR during infection, instead of a proximal 3’-UTR. This likely affects the loading of regulatory RNA-binding proteins or miRNA-RISC complexes, and thus translation.

The PhD project now aims at better understanding (1) the molecular mechanisms driving the isoform switch and translational regulation of [fill in] transcripts during infection, and (2) the impact of these changes on host cell viability, or their consequences on Listeria intracellular replication and dissemination.

* The identity of the regulator is confidential. It will be disclosed to applicants as well as to the doctoral school jury in due time.
(1) How is expression regulated during infection?

This part of the project aims at dissecting the molecular basis for regulation.

Using fusion with luciferase reporters, the PhD candidate will identify the cis-elements within alternative 3'-UTRs that condition the stability and/or translation of the transcripts during infection. Using isoform-specific FISH probes, he/she will also assess if the two forms of the transcripts are differently localised, in infected and non-infected cells.

Host trans-acting factors known to act alternative splicing, or binding 3'-UTR motifs present in the alternative isoforms, will be tested for their ability to regulate during infection, by knocking them down or out. In case this approach fails, the student will search for putative novel regulators binding to transcripts, by mRNA capture coupled with stable isotope labelling with amino-acids in cell culture (SILAC) (Butter et al. 2009) in order to quantify changes in association during infection. before eventually refining the contribution of candidate-specific miRNAs.

Last, using Lm mutant strains, other intracellular bacteria or purified effectors, the student will investigate whether the transcript stability and/or translation are actively controlled by Lm, and in particular by secreted bacterial effectors.

(2) How does function impact on intracellular bacterial infection?

To determine the role of during infection, the PhD candidate will assess a potential “pro-bacterial” or “anti-bacterial” effect by quantifying infectivity with different intracellular bacteria as well as wild-type or defective Lm mutants upon silencing or overexpression. To delineate which function of is involved, he/she will test if the silencing or overexpression of different functional partners, or the use of truncated versions of , are changing infection outcomes.

To identify the putative mRNA targets of that could impact infection, the student will compare occupancy on human transcripts between infected and non-infected conditions, by RNA-immunoprecipitation followed by sequencing (RIP-Seq) (Rissland et al. 2017). Incidentally, this strategy could provide insight into the mechanisms of post-transcriptional regulation of some transcripts identified in our previous –omics datasets. targets that might constitute novel candidate players in the control of Lm infection will be further validated and characterised.

(3) Possible related side-project in bio-informatics

In case of a keen interest for a mixed training in molecular biology and bio-informatics, the candidate may investigate if other alternative splicing isoforms are expressed during infection, by further mining our paired-end RNA-Seq and MinION datasets. The impact of these events on the translation of targets and on the outcome of infection would then be validated.

Faisabilité du projet de thèse (1/2 page maximum, en anglais)

The student will be hosted in our group Bacterial Infection & RNA Destiny, in the Functional Genomics section at IBENS, in a highly stimulating environment gathering all the expertise and equipment required for the success of the project. The candidate will be immersed in the multidisciplinary field of host-pathogen interactions, which is highly prone to the thriving of scientists with a broad scientific culture.

This project will rely on a wide range of approaches including bacteriology (Listeria genetics; infections), cell biology (immunofluorescence combined with RNA-FISH; siRNA and plasmid transfections; possibly genome editing), molecular biology and biochemistry (RIP-Seq, RNA capture, polyosome fractionation; RT-qPCR; western blots; click-chemistry; etc.), which will provide the PhD candidate with a versatility of skills and the appropriate means to answer his/her scientific questions. Except for gene editing, all of these approaches are perfectly mastered in our team and rely only on equipment available on-site. The group of Hervé Le Hir has implemented CRISPR-based genome editing in the Functional Genomics section; José Carlos Fernandez, an IE in our team, is currently getting trained for this technique.

Surprisingly, the team of Lionel Navarro in our institute, with whom we have been collaborating for four years on another project, has also recently observed that the homologue of Arabidopsis thaliana was involved in the host-pathogen crosstalk during the infection of plant tissues by the phytopathogen bacterium Pseudomonas syringae. Joined scientific discussion, expertise and tool sharing will be continued on this topic between our two teams, which should be a benefit for the progress of both projects.

In case a mixed wet-dry training is pursued, the PhD student will benefit from the infrastructure and diverse expertise of the members of the IBENS Computational Biology Centre. This transversal structure aims at reinforcing synergies between individual computational scientists in each team, by mutualising a potent computing cluster, providing training alongside specialised workshops and seminars, and stimulating regular exchanges on methodological issues. Computational biology analyses will rely on Python and R scripts, taking advantage of existing R packages and other relevant software. The dedicated analysis of processing changes will require benchmarking of existing tools, and further development in collaboration with the IBENS Genomics facility. Jupyter notebooks will be used to develop, annotate and share the code together with the result so as to ensure that scripts can be durably understood and used by different users. Any tool developed for this project will be implemented on a private GitHub, and made open upon publication.
Thèses actuellement en cours dans l’équipe
Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d’une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

<table>
<thead>
<tr>
<th>Nom et Prénom du doctorant</th>
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<th>Année de 1ère inscription</th>
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<td>Peron Cane Caroline (co-direction)</td>
<td>Nicolas Desprat (ENS, LPS) et Alice Lebreton</td>
<td>2015</td>
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Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.

