PhD project in the MMSB team "Molecular Ecology and Evolution of Phages" (Chevallereau's team)

Deciphering Prophage-Mediated Anti-Phage Defence Mechanisms in *Pseudomonas aeruginosa*

Background and Rationale: Bacterial infections remain a leading global cause of mortality, responsible for over 13 million deaths in 2019. While antibiotics have been the cornerstone of treatment, the rapid rise of antimicrobial resistance threatens their efficacy, posing a critical public health challenge. Phage therapy—the use of bacterial viruses (phages) to target multidrug-resistant bacteria—has emerged as a promising alternative. However, the evolution of phage resistance in bacteria risks replicating the challenges posed by antibiotic resistance, potentially undermining clinical outcomes.

Bacteria can resist phage infection via receptor incompatibility: a cell that either lacks or mutates the cell surface structures phages bind to will evade lysis. In addition, **bacteria can encode specialised defence systems** – protein(s) that act to prevent phage-induced lysis. These systems can operate through various mechanisms such as preventing DNA injection, degrading injected DNA/RNA, or inducing cell death before the infection cycle completes. Interestingly, prophages - phages that are integrated into bacterial genomes - have been recently recognised **as hotspots for defence systems** against competing phages. Prophages are well recognised as vehicles of horizontal gene transfer, and therefore may be **a core vector for the rapid dissemination of defence genes** within bacterial populations, which may pose a significant hurdle to the efficiency of phage therapy. Understanding how prophages mediate resistance against therapeutic phages is therefore crucial to anticipate the emergence of resistance in clinical isolates.

Preliminary Findings: In the ESKAPE pathogen *Pseudomonas aeruginosa*—a key target for phage therapy—we identified a prophage that confers resistance to a broad range of therapeutic phages. We discovered that this prophage provides resistance through two distinct mechanisms: it can either block the binding or disrupt the intracellular lifecycle of competing phages, suggesting that this prophage encodes multiple defence genes.

Objectives and workplan: This PhD project aims to **identify the genes and characterize the molecular mechanisms** underlying prophage-encoded anti-phage defence in *P. aeruginosa*. This project, structured in 3 tasks, uses **complementary and innovative approaches** combining genetic, proteomic, computational, biochemical and evolutionary methods for robust discovery.

- **Task 1. Identify the genetic determinants of prophage-mediated resistance**

The PhD candidate will implement two complementary approaches to identify the genes responsible for phage resistance. The construction of a transposon mutant library of the prophage will allow to select mutants that no longer provides their host with protection against therapeutic phages. In addition, the candidate will methodically fragment the prophage genome into a library of plasmids to determine the minimal genetic unit for resistance. Once the genes are identified, the candidate will seek to understand the molecular mechanism underlying resistance.

- **Task 2. Identify prophage-mediated defence mechanism**

The candidate will identify bacterial interactors of the phage defence proteins using global proteomic approaches (e.g., bacterial-two hybrid, proximity labelling and mass spectrometry). These results, combined with computational functional prediction (e.g. AlphaFold), will guide targeted experiments to identify the activity of the defence proteins and their host targets. Upon purification, the activity of the prophage proteins will be tested *in vitro* using biochemical assays such as enzymatic assays (DNase, protease, kinase, etc.) or binding assays to identify small ligands (e.g., nucleotides) and/or confirm their binding to host targets.

- **Task 3. Identify therapeutic phage proteins that trigger prophage defence mechanisms.**

The candidate will perform one-sided evolution experiments to generate and sequence mutant therapeutic phages which escape the prophage-encoded defence systems. Identified escape mutations will indicate which genes/proteins in the therapeutic phages are targeted by prophage defence proteins, to further elucidate the defence mechanisms. In addition, these results will demonstrate how quickly defences can be evaded by virulent phages.

This project will **advance fundamental knowledge** by revealing novel mechanisms of anti-phage defence, contributing to our understanding of bacterial-phage coevolution. In addition, it will **inform phage therapy** by identifying potential barriers and strategies to overcome them, improving the design of therapeutic phage cocktails.

Supervision: The candidate will be supervised by Dr. Anne Chevallereau (expertise in phage biology and bacterial genetics) together with Dr. Josie Elliott, postdoc in the team. In addition, the candidate will collaborate with teams expert in biochemistry at MMSB and with the PSF platform.