Project 3

Deciphering Fis-mediated genome architecture and xenogeneic regulation in *Legionella pneumophila*

Overview:
This project aims to elucidate the novel functions of the nucleoid associated proteins (NAPs) Fis1, Fis2 and Fis3, as xenogeneic regulators in the human pathogen *Legionella pneumophila*. Traditionally recognized to play a crucial role in regulating DNA topology and gene expression, notably those involved in virulence in many pathogens, our recent finding also revealed that *Legionella* Fis protein may play a role in the integration and expression of horizontally acquired foreign DNA. By integrating genome-scale approaches with biomolecular and genetic analyses, this research aims to uncover how Fis DNA binding and bending activities contribute to the remodeling of chromosome architecture, drive mobile genetic elements (MGEs) dynamics and expression, and ultimately influence phenotypic diversity and evolutionary outcomes.

Background and Rationale:
Nucleoid-associated proteins (NAPs) play a crucial role in bacterial chromosome architecture, DNA topology and influence DNA-related processes such as replication, transcription and DNA repair. By binding DNA in a specific or non-specific manner, NAPs such as H-NS, Fis, IHF, HU, and Dps modify chromatin structure, modulate RNA polymerase accessibility and activity and thus impact gene regulation (1). Beyond these functions, accumulating evidence suggests that NAPs also regulate the integration and expression of foreign genes carried by MGEs (2–5).
MGEs, such as plasmids, prophages, integrative conjugative elements (ICEs) and transposons, drive the bacterial evolution, allowing bacteria to acquire new traits, such as antibiotic resistance, new metabolic functions or virulence traits. However, integrating foreign genes can introduce a genetic burden, disrupt regulatory networks and strongly impaired fitness. To counterbalance these effects, bacteria have evolved sophisticated mechanisms, often mediated by NAPs like H-NS—to silence newly acquired genes (xenogeneic silencing) (6, 7) or guide MGEs integration such as transposon into non-essential genomic regions (2).
Our research focuses on the roles of NAPs, particularly the Fis family proteins, in modulating DNA topology and gene expression in the human pathogen *Legionella pneumophila*. Unlike most bacteria, which possess a single Fis protein, *L. pneumophila* has three distinct homologs—Fis1, Fis2, and Fis3—each with diverging evolutionary functions. Our recent findings indicate that these proteins regulate virulence gene expression in a sequential and complementary manner, making them key players in *Legionella* pathogenicity (8). Another remarkable feature regarding NAPs in *Legionella* is that, despite harboring numerous MGEs, this bacterium does not possess H-NS protein, the xenogeneic silencer. This raises the possibility that *Legionella* uses an alternative mechanism to control the expression of horizontally acquired genes. Our RNA-seq data and preliminary functional analyses support this hypothesis, showing that Fis proteins repress a significant number of MGEs-encoded genes, including those involved in excision and integration, potentially acting as alternative regulators of foreign DNA.
This project aims to elucidate the unique roles of *L. pneumophila* Fis proteins in genome organization and MGEs regulation, providing new insight into how this bacterium maintains genomic integrity while balancing horizontal gene acquisition.

Research strategy and methodology.
This project is organized into three integrated axes:
In the first axis, Chromatin immunoprecipitation followed by sequencing (ChIP-seq) will be used to generate genome-wide binding profiles for Fis1, Fis2, and Fis3. This approach will allow us to identify regions of the chromosome where Fis proteins are enriched, particularly focusing on promoters of MGEs-encoded genes and known integration sites. We will compare the binding profiles under various growth conditions and in different *L. pneumophila* strains, including those with differing MGEs content, to assess how environmental and genomic factors influence Fis occupancy.
In the second axis, 3C-seq (Chromosome Conformation Capture sequencing)-based method will be employed to examine the 3D-structure of the *L. pneumophila* chromosome. By comparing the chromosomal architecture in wild-type and Fis-mutant strains, we will be able to detect the chromosomal loops and higher order domains that are mediated by Fis proteins, particularly around regions that harbor MGEs. The integration of 3C-seq with the ChIP-seq binding maps will clarify how Fis proteins may bring MGEs regions into proximity with other genomic areas or spatially segregate MGEs from the rest of the genome, thereby modulating their expression.
In the final axis, we will integrate the previous findings with gene expression data. Digital droplet PCR (ddPCR) will quantify MGEs excision/integration rates and gene expression in wild-type and *fis*-mutant strains, thereby clarifying whether Fis-mediated repression influences not only gene expression but also MGEs dynamics. Furthermore, by using different biomolecular interaction techniques (EMSA, footprinting assay, ITC, thermophoresis) will achieve a depth characterization of the nature of the interactions of the Fis proteins and decipher the spatial and functional interplay between NAPs and MGEs.

Expected Outcomes and Significance:
By deciphering the molecular mechanisms governing MGEs dynamic and expression, this project will provide a deeper understanding of bacterial genome plasticity and its impact on pathogenicity and adaptation, explaining the ecological success of certain *L. pneumophila* clones in infecting human hosts. The findings could also reveal previously unknown regulatory strategies in *L. pneumophila* and highlight the evolutionary importance of NAPs in shaping bacterial genomes.

1. Dame RT, Rashid F-ZM, Grainger DC. 2020. Chromosome organization in bacteria: mechanistic insights into genome structure and function. Nat Rev Genet 21:227–242.
2. Cooper C, Legood S, Wheat RL, Forrest D, Sharma P, Haycocks JRJ, Grainger DC. 2024. H-NS is a bacterial transposon capture protein. Nat Commun 15:7137.
3. Dorman CJ, Kane KA. 2009. DNA bridging and antibridging: a role for bacterial nucleoid-associated proteins in regulating the expression of laterally acquired genes. FEMS Microbiol Rev 33:587–592.
4. Singh K, Milstein JN, Navarre WW. 2016. Xenogeneic Silencing and Its Impact on Bacterial Genomes. Annu Rev Microbiol 70:199–213.
5. Flores-Ríos R, Quatrini R, Loyola A. 2019. Endogenous and Foreign Nucleoid-Associated Proteins of Bacteria: Occurrence, Interactions and Effects on Mobile Genetic Elements and Host's Biology. Comput Struct Biotechnol J 17:746–756.
6. Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H, Libby SJ, Fang FC. 2006. Selective silencing of foreign DNA with low GC content by the H-NS protein in Salmonella. Science 313:236–238.
7. Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M, Hinton JCD. 2006. H-NS mediates the silencing of laterally acquired genes in bacteria. PLoS Pathog 2:e81.
8. Andréa C, Bresson J, Ginévra C, Vianney A, Bailo N, Chapalain A, Attaiech L, Picq K, Ranquet C, Nasser W, Doublet P, Kay E. 2024. Fis family members synergistically control the virulence of *Legionella pneumophila*. bioRxiv 2024.09.25.615009.

Supervisor(s) name(s), Affiliation(s), eMail address(es) for contact:

Elisabeth KAY, Centre International de Recherche en Infectiologie (CIRI), Equipe « Pathogénie des Légionelles » dirigée par Patricia DOUBLET/Sophie JARRAUD

elisabeth.kay@univ-lyon1.fr

Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

- [x] Host-pathogen interactions;
- [ ] Mechanisms of bacterial resistance and cancer onsets;
- [ ] Neurodegenerative and autoimmune diseases;
- [ ] Translational research in prevalent diseases;
- [ ] Physiology and ecology;
- [ ] Neurosciences and cognition.