MASTER « *In Silico* Drug Design »

1ère année

PROPOSITION DE STAGE
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Titre du stage : Deciphering the molecular interaction between Mfd and UvrA in *E.coli*

Description du sujet (quelques lignes):

Human gut microbiota clusters at least 1,000 different species of known bacteria that attain more than 3 million genes and weigh up to 2 kg per adult. If one third of human microbiota is common to most people, two thirds are specific to every one of us, thus behaving like a health colonic ID card. A balanced composition of microbiota associates with healthy patients whilst reduction in bacterial biodiversity results in dysbiosis and correlates with defective host-microbial interactions and diseases like obesity or Inflammatory Bowel Disease. Among abnormal host-bacteria interactions, secretion of reactive chemicals such as nitric oxide (NO) induces bacteria DNA damage and gene mutation. NO-induced damage to the DNA template interferes with gene transcription and thereby compromises bacterial viability.To combat this, bacteria possess mechanisms to limit the negative effects where Mfd (Mutation Frequency Decline) plays a pivotal role as it is an evolutionarily-conserved protein that was shown to mediate DNA repair in *Escherichia coli* and *Helicobacter pylori*. Furthermore, MFD has been associated with the development of antibiotic resistance in *Campylobacter jejuni* and *H. pylori*. Freshly, a role for Mfd in bacterial virulence is to be reported as N. Rama Rao and co-workers from the Micalis Institute (INRA of Jouy en Josas) have shown that Mfd is required for survival of pathogen bacteria in their host, by protecting them against DNA damage following NO exposure. As Mfd is widely conserved in the bacterial kingdom, these data highlight a mechanism that may be used by a large spectrum of bacteria to overcome the host immune response.

Mfd is a Transcription Repair Coupling Factor, as TRCF is the product of mfd gene, widely conserved in almost all bacteria and absent in higher eukaryotes. Mfd is a large multi-functional protein with a complex structure/function relationship comprising a compact arrangement of eight structured domains, clustered in functional modules and connected by flexible linkers. Through intricate intramolecular and interdomain conformational change, Mfd adopts distinct conformations compatible with its numerous functions, such as RNAP binding, ATPase and DNA translocase enzymatic activities. Several lines of evidence showed that Mfd needs to go through a dynamic remodeling of its domains to be able to bind to RNAP, then to accommodate and hydrolyze ATP, and finally to recruit UvrA, a partner of the NER complex. Indeed, Mfd structure of E. coli evidences a repressed conformation, incompatible with a full ATP-ase activity or the recruitment of UvrA.

This internship aims at characterizing the later step of this cycle, namely the interaction of Mfd with UvrA in *E. coli*. The internship will take place in MaIAGE, at INRA in Jouy-en-Josas, and in close collaboration with the microbiologist from PIMs. MaIAGE lab gathers mathematicians, computer scientists, bioinformaticians and biologists to tackle problems coming from microbiology. The addressed questions concern metabolic and signaling processes at molecular and cellular levels. During his/her internship, the student will learn how to model protein/protein interaction, identify and map the residues located at the putative interface and analyze their conservation level. Possibly this work could be extended to the modeling of similar complexes in *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* bacteria. Accordingly, addressing this point will require the subsequent use of homology modeling.

In terms of molecular modeling, this internship should allow the use of numerous tools: PyMOL, Modeller, Rosetta, etc. and database mining such as pdb, uniprot etc. Moreover, the close discussion with our microbiology partners will be an opportunity to be part of tackling current challenges.
