MOLECULAR BASIS OF TRANSCRIPTIONAL INTERFERENCE BY CROSS-TALK BETWEEN TRANSCRIPTION FACTORS AND ITS EFFECT ON BACTERIAL FITNESS.

Fundamental processes in all living systems are mostly regulated at the level of gene expression, which provides the adaptation to the rapidly changing environment to increase the chances of the survival. This control is multi-layered, but largely operates at the level of transcription, with possible outputs of gene expression activation, inhibition or complete silencing. The regulation of gene expression and coordination of genetic networks, relies on transcription factors (TF). Those relatively small proteins are able to interact with their DNA target sites, as well as RNA polymerase and many other molecules, collectively to provide host adaption to a broad range of environments, including response to stresses and the presence of dynamically changing conditions. In transcriptional control, each gene is regulated directly or indirectly by one or more TFs. A group of genes directly controlled by the same TF forms a regulon, which together with other sets of interacting regulons generates a transcriptional genetic network. The action of each TF is determined by binding to a specific DNA sequence/site referred as sequence motifs. Though there are some highly specific TFs dedicated to regulate a single gene or operon (gene target), some TFs are tuned to operate less specifically to affect large groups of genes (regulons). Such mode of action is usually associated with the level of specificity achieved in recognition of their DNA binding sites. In addition, most TFs are also able to recognize secondary target sites (nonspecific=off-target), making the regulatory systems even more complex. This is why, such a multi-layered pyramid of cellular regulatory networks is prone to a regulatory cross-talk, in conditions where certain transcription factor could affect transcription outside its target regulon (positively or negatively) with potentially disastrous consequences for the cell. This process of transcriptional cross-talking is still not well understood and its effect underestimated. Knowledge on complete transcriptional genetic network on individual microorganism does not exist, though *Escherichia coli* K-12 MG1655 has one of the most widely studied genomes, but still approximately 30% of its genes is not characterized including about 50–80 TFs.

The main objective of this proposal is to determine the molecular basis of the transcriptional cross-talking between bacterial transcription factors: C regulatory protein of restriction-modification system and RacR repressor of crypthic prophage. Recently, we detected that the C protein off-target action within the Rac prophage region of *E. coli* genome affected the bacterial cell viability. The proposed study will be a follow up on a detailed molecular mechanism of the C protein nonspecific DNA interaction and its implication on bacterial fitness. In addition, we also aim to determine the new off-target sites for the model C protein using novel, systemic *in silico* comparative genomic approach. It is likely that more cross-talks affecting cell fitness will be determined.

We expect our results to provide insight into, and lay groundwork for further studies of regulatory networks, transcription activation/repression and molecular ecology of mobile operons. This work will help to understand the basic mechanisms responsible for adaptation of bacteria to the changing environment, for bacterial genomes shaping and formation of molecular barrier in the horizontal gene transfer.