Title of the PhD project:

Regulation of gene expression coupled to cell growth and division in bacterial adaption processes.

PhD Supervisor

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Laboratory : LCQB, UMR 7238
Title of the team : Biologie des Génomes
Team leader (*if different) : Gilles FISCHER*
Doctoral School : ED 515, Complexité du vivant

Overview of the scientific projects of the team

Our research projects aim at understanding the biology of prokaryotic and eukaryotic genomes. We are combining experimental approaches based on molecular genetics and functional genomics to quantitative explorations of genome sequences requiring bio-informatic skills and the development of new tools. We are interested by the relationships between replication/recombination, the gene organization along chromosomes and their effect on gene expression in both yeast and bacteria. In yeast, we study the evolution of gene content and all mutational mechanisms leading to chromosomal rearrangements, variations of nucleotide composition and sequence polymorphisms at different scales, from cells to populations, and from strains to species. In bacteria, we are studying how chromosome structure can affect gene expression regulation as a function of the DNA replication cycle using single cell measurements.

Main publications since January 1er, 2016

- Qing Zhang, Elisa Brambilla, Rui Li, Hualin Shi, Marco Cosentino Lagomarsino, Bianca **Sclavi** (2020) A decrease in transcription capacity limits growth rate upon translation inhibition. mSystems, mSystems 5:e00575-20. https://doi.org/10.1128/mSystems.00575-20.

- Crozat E, Tardin C, Salhi M, Rousseau P, Lablaine A, Bertoni T, Holcman D, **Sclavi** B, Cicuta P, Cornet F. (2020) Post-replicative pairing of sister ter regions in Escherichia coli involves multiple activities of MatP. Nat Commun. 2020 Jul 30;11(1):3796. doi: 10.1038/s41467-020-17606-6.

- Yousuf M, Iuliani I, Veetil RT, Seshasayee ASN, **Sclavi** B, Cosentino Lagomarsino M. (2020) Early fate of exogenous promoters in E. coli. Nucleic Acids Res. 2020 Mar 18;48(5):2348-2356. doi: 10.1093/nar/gkz1196. PMID: 31960057

- Wurihan, W., GeZi, G., Brambilla, E., Wang, S., Sun, H., Fan, L., Shi, Y., **Sclavi**, B.,*, Morigen M.* (2018) DnaA and LexA proteins regulate transcription of the uvrB gene in Escherichia coli: the role of DnaA in the control of the SOS regulon, Front. Microbiol. | doi: 10.3389/fmicb.2018.01212

- Bury-Moné, S., **Sclavi**, B. Stochasticity of gene expression as a motor of epigenetics in bacteria: from individual to collective behavior, Research in Microbiology (2017) 168(6), 503-514

PhD Co-Supervisor

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Title of the team : Statistical physics of cells and genomes.
Team leader *(if different) :*
Doctoral School :

Overview of the scientific projects of the team

Main publications since January 1er, 2016

Gabriele Micali, Jacopo Grilli, Matteo Osella, **Marco Cosentino Lagomarsino** Concurrent processes set E. coli cell division Science Advances 4:11 eaau3324 2018

Clotilde Cadart, Sylvain Monnier, Jacopo Grilli, Rafaele Attia, Emmanuel Terriac, Buzz Baum, **Marco Cosentino Lagomarsino**, Matthieu Piel Size control in mammalian cells involves modulation of both growth rate and cell cycle duration Nature communications 9

Q Zhang, F Bassetti, M Gherardi, **M Cosentino Lagomarsino** Cell-to-cell variability and robustness in S-phase duration from genome replication kinetics Nucleic Acids Research, 45(14):8190-8198. doi: 10.1093/nar/gkx556 2017

Matteo Osella, Sander J. Tans, **Marco Cosentino Lagomarsino** Step by Step, Cell by Cell: Quantification of the Bacterial Cell Cycle, Trends in Microbiology, 25 4 250-256 2017

AS Kennard, M Osella, A Javer, S Tans, P Cicuta, **M Cosentino Lagomarsino** Individuality and universality in the growth-division laws of single E. coli cells. Phys. Rev. E, 93, 012408 2016.

Doctoral Project

Title: Regulation of gene expression coupled to cell growth and division in bacterial adaption processes.

Abstract :

The DnaA protein can act as the regulatory link between the timing and level of gene expression and the different phases of the bacterial cell cycle. The cooperative binding of the DnaA protein to the origin of replication leads to DNA opening and the assembly of the replication forks. But DnaA is also a transcription factor, a highly connected node in the network of genes coding for proteins required for DNA replication and the repair of DNA damage. However, a description of the regulation of gene expression by DnaA as a function of the cell cycle is still lacking. The interdisciplinary approach used here will consist in the measurement in gene expression and cell growth parameters in real time at the single cell level by using microfluidics coupled to microscopy. This quantitative data will then be used for the creation of both stochastic and coarse-grained mathematical models that can be used to test different possible hypothesis on the regulatory links between gene expression and cell growth and division that permit rapid cellular adaptation to changes in growth rate.

Context and objective :

Little is known about how gene expression is coupled to the regulation and timing of specific events of the cell cycle, such as initiation of DNA replication or cell division, particularly in fast growing bacteria such as *E. coli*. These processes are regulated to maintain a robust control of cell size despite the stochasticity of biochemical reactions in vivo and of the frequent delays in the DNA replication process due to replication forks encountering transcription complexes or DNA damage. Recent work has shown that cells add a constant size before each cell division, independently of the cell size at birth[1,2]. This results in a narrow distribution of cell sizes in a bacteria population that is optimal for a specific nutrient-imposed growth rate. Different mathematical models have been proposed exploring different aspects of cell growth control, from the amount of resources dedicated to protein and ribosomes synthesis, to the synthesis of surface components, membrane and cell wall, in relation to volume growth[3]. However, the molecular mechanisms behind these observations remain to be identified.

Despite the fact that completion of the DNA replication program is required before cell division can take place, the role of the regulation of the DNA replication in cell size control as cells adapt to different growth rates remains to be established. The main factor regulating both the initiation and the rate of elongation of DNA replication is the DnaA protein[4]. Its activity depends on its nucleotide bound state, the ATP bound form being the active one for origin recognition and activation. The hydrolysis of ATP to ADP decreases its activity to avoid re-initiation of DNA replication within the same cell cycle. Furthermore, DnaA is also a key transcription factor regulating the expression of its own gene as well as the gene for ribonucleotide reductase (RNR), the enzyme providing the dNTPs required for DNA synthesis, thus influencing the elongation rate of the replication forks[5]. In previous studies we have shown that the autoregulation of the expression of the *dnaA* gene takes place via both positive and negative regulation[6] and that the positive and negative regulation of the RNR gene

expression by DnaA is required for the correct timing and amplitude of induction during the cell cycle[7]. Finally, we have shown that DnaA also regulates the expression of DNA repair enzymes so that it may be proportional to the amount of DNA in the cell to efficiently clear the way for the replication forks to reach completion of the genome synthesis[8].

One of the major challenges in this field has been to quantify the changes in DnaA-ATP activity in vivo in real time. To address this problem, we have developed a set of reporters of gene expression using a gene for a fluorescent protein under control of a promoter that can be differentially regulated by DnaA-ATP. Using a microfluidic device coupled to microscopy imaging these strains can be used to follow the changes in gene expression rate during the cell cycle by measuring fluorescence in real time at the single cell level.

The project presented here will be the continuation of the PhD thesis of Ilaria Iuliani, which is being developed thanks to the longstanding collaboration between her two thesis advisors whose expertise resides in experimental biology (BS) and theoretical physics (MCL). Ilaria Iuliani is a PhD student with a background in physics in the 4th and final year of her PhD in the Big team at the LCQB. She has established both the experimental and image and data analysis protocols to obtain data on the activity of different promoter variants as a function of the cell cycle. Thanks to her results, she has been able to quantify for the first time the effect of the DnaA-dependent promoter regulatory elements that lead to an oscillatory pattern of gene expression that is coupled to both the DNA replication and cell division programs. In collaboration with Marco Cosentino Lagomarsino she is currently using these quantitative data to develop different stochastic mathematical models that will allow us to test our current hypothesis and predict the effect of novel mutations. More specifically, the changes in gene expression from the different promoter variants should all be consistent with a cell cycle dependent change in DnaA activity. Mathematical models of the transcription regulation by DnaA allow Ilaria to obtain an estimate how the activity of DnaA changes to result in the experimentally observed patterns.

The aim of this new PhD project is to use the same approach, coupling quantitative biology with mathematical modeling, to test the hypothesis that have emerged from Ilaria's results by making mutations on the endogenous promoters for the DnaA gene as well as its target genes. Oana Ilioaia, engineer in our team, has recently set up a Crispr protocol to obtain the first set of mutant strains. These and future mutant strains will be used to characterize the DNA replication properties in the absence of specific regulatory elements. This will be done at first at the population level, by measuring the number and the synchrony of active replication origins by flow cytometry, and then by single cell fluorescence where different sites along the genome have been tagged by fluorescent proteins, both techniques that have already been used successfully in our lab. The change in DnaA activity in these mutant strains and upon bacterial adaptation to different growth conditions and in response to DNA damage will be measured by the real time single cell gene expression reporter assay. Preliminary data by our two groups (BS and MCL) and colleagues in Cambridge, UK on the time evolution of cellular adaptation following a shift in growth rate, for example, have revealed the presence of interesting transient states that merit further investigation[9]. These results will allow us to detail the mechanisms that permit the cell to rapidly and efficiently regulate its gene expression program to adapt the DNA replication process to different growth conditions, notably to changes in growth rate depending on the availability of nutrients and to the presence of DNA damage.

Justification of suitability for *i-Bio*:

This project is the result of a longstanding collaboration between an experimental biologist, Bianca Sclavi, at the LCQB, UMR 7238 of Sorbonne Université and a theoretical physicist, Marco Cosentino Lagomarsino, at IFOM/University of Milan. This project requires the tight interplay of these two approaches by the acquisition of quantitative data in real time at the single cell level revealing the stochasticity, population heterogeneity and robustness of the adaptation process. These data can be used to generate and test mathematical models describing the gene regulatory networks and complex temporal evolution of biological systems as they adapt to changing environments and stressful DNA-damaging conditions.

Role of each supervisor / skills provided:

The two supervisors of this thesis project have in the past trained several students who have a background in physics to work on biological questions with experimental biologists. The students have been able to learn both the experimental and theoretical side of this research approach and have been able to make significant contributions to our shared research programs.

Bianca Sclavi: Training in molecular biology and genetics of *Escherichia coli* to produce fluorescent reporter strains and Crispr mutant strains. Microfluidics and microscopy for in vivo, real-time imaging. Fluorimetry, flow cytometry and quantitative PCR. Data analysis using MatLab and R.

Marco Cosentino Lagomarsino: mathematical models and model-guided data analysis, stochastic dynamics of both gene expression and cell growth and division processes.

Profile of the desired student:

The desired student should be enrolled in or have completed a master program in physics or in an interdisciplinary master program. They should be curious about biology and wanting to gain expertise in quantitative biology experimental approaches as well as the development of methods to analyze large complex experimental datasets so that they can be used to propose and test predictive models.

References

1. Ho, P.-Y. & Amir, A. Simultaneous regulation of cell size and chromosome replication in bacteria. *Front Microbiol* **6**, (2015).
2. Taheri-Araghi, S. *et al.* Cell-size control and homeostasis in bacteria. *Curr. Biol.* **25**, 385–391 (2015).
3. Westfall, C. S. & Levin, P. A. Bacterial Cell Size: Multifactorial and Multifaceted. *Annu. Rev. Microbiol.* **71**, 499–517 (2017).
4. Katayama, T., Kasho, K. & Kawakami, H. The DnaA Cycle inEscherichia coli: Activation, Function and Inactivation of the Initiator Protein. *Front Microbiol* **8**, 2496 (2017).

5. Odsbu, I., Morigen & Skarstad, K. A reduction in ribonucleotide reductase activity slows down the chromosome replication fork but does not change its localization. *PLoS One* **4**, e7617 (2009).
6. Saggioro, C., Olliver, A. & Sclavi, B. Temperature-dependence of the DnaA-DNA interaction and its effect on the autoregulation of dnaA expression. *Biochem. J.* **449**, 333–341 (2013).
7. Olliver, A., Saggioro, C., Herrick, J. & Sclavi, B. DnaA-ATP acts as a molecular switch to control levels of ribonucleotide reductase expression in Escherichia coli. *Mol. Microbiol.* **76**, 1555–1571 (2010).
8. Wurihan, *et al.* DnaA and LexA Proteins Regulate Transcription of the uvrB Gene in Escherichia coli: The Role of DnaA in the Control of the SOS Regulon. *Front Microbiol* **9**, 1212 (2018).
9. Panlilio, M. *et al.* Threshold accumulation of a constitutive protein explains E. coli cell division behavior in nutrient upshifts. *bioRxiv* 2020.08.03.233908 (2020) doi:10.1101/2020.08.03.233908.