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

Bianca Sclavi, Biology of Genomes team (BiG), LCQB, UMR 7238, CNRS, Sorbonne Université, 75005 Paris, France
Marco Cosentino Lagomarsino, Statistical physics of cells and genome, IFOM Foundation / University of Milan, Milan, Italy

Despite many years of work on the model organism *Escherichia coli*, still 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. 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 (Ho and Amir, 2015; Taheri-Araghi et al., 2015). 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 (Westfall and Levin, 2017). 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 (Katayama et al., 2017). 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 (Odsbu et al., 2009). In previous studies we have shown that the autoregulation of the expression of the *dnaA* gene takes place via both positive and negative regulation (Saggioro et al., 2013) 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 (Olliver et al., 2010). 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 (Wurihan et al., 2018).

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, defended in December 2021, which is the result of a longstanding collaboration between her two thesis advisors whose expertise resides in experimental biology (BS) and theoretical physics (MCL). Ilaria Iuliani began her PhD with an undergraduate degree in physics and a Master degree on complex systems and thanks to her PhD project developed an expertise in bacterial physiology and microscopy. During her PhD, Ilaria has established both the experimental and image and data analysis protocols required 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 leading to an oscillatory pattern of gene expression. Furthermore, via a mathematical analysis of these data she has been able to show the presence of a strong coupling between the oscillations in DnaA activity, changes in cell volume and the cell division programs (manuscript in preparation). In the lab of Marco Cosentino Lagomarsino she is currently using these results 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.

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 the BiG team at the LCQB, 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 these 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 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. Recent data by our two groups (BS and MCL), in collaboration with colleagues in Cambridge, UK, on the time evolution of cellular adaptation following a shift in growth rate, have revealed the presence of interesting transient states in both growth rate and cell size. These results have allowed us to test different possible models for the control of expression of a cell division factor (Panlilio et al., 2021). These observations merit further investigation and allow us to formulate specific hypothesis about the mechanisms involved in the timing of the changes in DNA replication and the control of cell division and cell size. The results obtained during the PhD project proposed here will allow us to detail the molecular 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.

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