Research Project Proposal for Master's Degree March 31, 2017

Workplace: Single Molecule Microbiology laboratory | ITQB NOVA | Oeiras, Portugal

Supervisor: Dr. Zach Hensel

Topic: Mechanism of cell-cycle-dependent gene regulation using single-molecule microscopy of bacteriophage λ transcriptional repressors Cro and CI

Background

The proteins Cro and CI from the bacteriophage λ bind specifically and non-specifically to the *E. coli* chromosome to regulate transcription. Cro and CI form a mutual repression genetic network that determines the fate of *E. coli* cells infected by bacteriophage λ, an important model system for microbial molecular and synthetic biology with analogues in higher organisms.

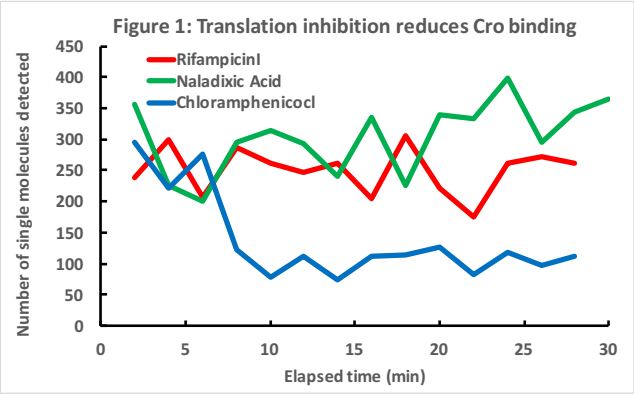
Previous experiments showed that Cro exhibits cell-cycle-periodic oscillations in gene expression. Computer simulations showed that this behavior was not consistent with existing models. Experimental observations were reproduced by adding transient changes in the rate of specific DNA unbinding associated with DNA replication. DNA replication is associated with changes in DNA supercoiling that may affect Cro binding.

Preliminary single-molecule tracking data show that non-specific DNA binding by Cro can be weakened by inhibiting translation. Translation inhibition is known to affect DNA compaction (and possibly supercoiling) in *E. coli*. Our emerging hypothesis is that local DNA supercoiling associated with DNA replication affects Cro specific and/or non-specific binding in a way that can contribute to oscillatory gene expression. It is possible that CI will show similar behavior.

Objectives

- Reproduce preliminary data: Perform single-molecule microscopy (PALM) to detect Cro molecules bound to DNA in cells treated by drugs affecting DNA conformation; image DNA-intercalating dyes to observe DNA conformation
- Engineer constructs for inducible, recombinant expression of fluorescently labeled Cro and CI as well as DNA topoisomerases
- Implement hardware/software for fast single-molecule microscopy required for imaging rapidly diffusing proteins
- Develop methods for fluorescent labeling of proteins in living *E. coli* cells using SNAP and/or HALO ligands

An individualized work plan will be developed based upon two or more of these objectives based upon the interests, skills, and career goals of the student.



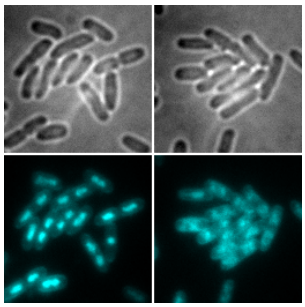


Figure 2: DNA compaction after translation inhibition (left) compared to untreated cells (right)