A Single-Cell View of Multiple Antibiotic Resistance in *Escherichia coli*

Cˆlin C. Guet[1], Luke Bruneaux[1], Panos Oikonomou[1], Max Aldana[2], Philippe Cluzel[1]

Short Abstract — The chromosomal *mar* operon is responsible for the *m*ultiple *a*ntibiotic *r*esistance phenotype in *Escherichia coli*. This operon is composed of a repressor, *marR,* and an activator, *marA*. MarA governs the expression of the efflux pump AcrAB-TolC, which is the main determinant of multi drug resistance. The genetics of multiple antibiotic resistance has been studied solely at the population level. Single cell studies of the dynamics of the *mar* operon are necessary in order to understand cellular variability of resistance to antibiotics. Using the fluorescent protein Venus-YFP, we monitor in real time the activity of the *mar* promoter in single cells across micro-colonies. This approach enables us to construct genealogies of promoter activities across several generations. We determine the activity of the *mar* promoter for different values of the inducer salicylate. We find that in response to a steady level of inducer, *mar* promoter activity is widely heterogeneous across genetically identical daughter cells. Often different promoter activity levels are maintained and inherited for several generations. We find that heterogeneity in promoter activity varies with inducer level. The *mar* promoter activity is homogeneous throughout colonies in a *ΔmarR* mutant strain.

I. Background

"In many ways, the fight against antibiotic resistance is already lost; preventing bacterial disease requires thoughtful new approaches" [1]. Multiple antibiotic resistance in pathogenic bacteria is a main threat to public health. The ***m***ultiple ***a***ntibiotic ***r***esistance or *mar* phenotype involves several transcriptional regulators that orchestrate the control of influx and efflux of toxic chemicals from the cell [2]. The activator MarA modulates the expression levels of one of the largest regulons in *Escherichia coli*. Our present knowledge of the *mar* system comes solely from experiments performed on populations of cells [3]. In order to accurately describe the dynamics of the underlying genetic networks as well as the cell-to-cell variability of the resistance phenotype, single cell studies become essential [4]. The *mar* system is conserved across many species of bacteria and the presence of few resistant cells in an otherwise genetically identical population of pathogenic bacteria can have major consequences for public health.

II. Results

We clone the fluorescent reporter gene Venus-YFP downstream the promoter of the *mar* operon on a very low copy plasmid, in order to measure the activity of the *mar* promoter. Using a soft lithography method, we grow single cells of *Escherichia coli* into linear micro-colonies. The linear micro-colonies enable us to reliably construct genealogies across 5-6 generations. We induce the cells with various levels of the inducer salicylate and monitor the activity of the *mar* promoter in single cells across several generations. Surprisingly, we find that upon steady salicylate induction, the *mar* promoter exhibits heterogeneous activity states across a genealogy tree. Interestingly, these activity states are being inherited across several generations and the levels of heterogeneity appears to change with salicylate levels. This epigenetic phenotype is abolished in a *ΔmarR* mutant strain as well as in a *ΔmarR ΔmarA* mutant strain. When we average out our single cell measurements into population level measurements, previous population induction dynamics are recovered [5].

III. Conclusions

The gene expression dynamics of multiple antibiotic resistance in *Escherichia coli* has been studied only at the level of population of cells. Population level studies have so far masked any heterogeneity in the gene expression of the *mar* system. By contrast, at the single cell level we find that the activity of the *mar* promoter is heterogeneous across single cells and that this phenotype can be inherited throughout several generations. Our study reveals the presence of nongenetic inheritance patterns in the activity of the *mar* promoter which controls multiple antibiotic resistance genes in bacteria.

Acknowledgements: Supported by a Chicago Biomedical Consortium catalyst grant. C.C.G. was supported by the Yen fellowship and Chicago Biomedical Consortium catalyst grant.

[1]Institute for Biophysical Dynamics and The James Franck Institute, The University of Chicago, Chicago, IL 60637, USA
[2]Instituto de Ciencias Fisicas, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico

References

[1] C.F. Amabile-Cuevas, *American Scientist* **91**, 138 (2003).
[2] X.Z. Li and H. Nikaido, *Drugs* **64**, 159 (2004).
[3] M.N. Alekshun and S.B. Levy, *Trends Microbiology* **7**, 410 (1999).
[4] T.T. Le, S. Harlepp, C.C. Guet, K. Dittmar, T. Emonet, T. Pan and P. Cluzel, *PNAS* **102**, 9160 (2005).
[5] S.P. Cohen, S.B. Levy, J. Foulds and J.L. Rosner *J Bact* **175**, 7856 (1993).