Evolution of antibiotic responses in complex dynamic settings

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***Short Abstract* — Microbial communities in their natural environments are remarkably dynamic and heterogeneous. However, we still lack a framework to predict how regulatory circuits evolve to control microbial behavior in such complex environments. Here, we use single-cell and microcolony microfluidic experiments to develop a mathematical model of the dynamics of bacterial cell responses, which captures the phenotypic diversity generated during antibiotic exposures. Then, we apply this model to analyze the short-term evolution of drug responses in continuous-culture evolution experiments. We show that loss of regulation of resistance, which is frequently observed in the clinic, can be favored during periodic exposure to large doses of antibiotics.**

***Keywords* — antibiotic resistance, biofilms, cell responses, gene regulation, dynamics, optimization.**

I. Cell responses are dynamic and heterogeneous

Microbial communities in their natural environments harbor the coexistence of diverse phenotypes and complex behaviors at the population level. Antibiotic responses are especially dynamic, subject to strong selective pressures, and undergo significant evolution in adaptation to new environments such as clinical settings. The dynamics of cellular responses is controlled by gene regulatory circuits, and therefore evolves according to the specific demands of its environment. Despite advances in understanding gene regulation at the molecular level, we still lack a framework to describe how regulatory circuits evolve to control microbial behavior. Here, we test the hypothesis that the noisy dynamics of cellular processes ultimately determines population behavior and the evolution of cell responses.

Using single-cell [1] and microcolony microfluidics [2], we show that, upon sudden exposure to a large drug dose, remarkable phenotypic diversity arises in resistant bacterial populations, with only a subpopulation being able to induce resistance appropriately and survive. We have found this to be a general feature of cellular responses in bacteria, caused by either the noisy dynamics of transcription regulation or by the reorganization of chemical gradients and growth patterns across bacterial colonies.

II. Evolution of responses in dynamic environments

Although it is known that the short-term evolution of microbes in new environments tends to favor mutations in regulatory pathways that adapt cell responses to new demands, it is not clear how specific dynamic regimens affect this evolution, or which mechanisms are commonly used by the cell to achieve new regulation strategies.

A. Mathematical model recapitulates dynamics and spatial structure of antibiotic responses

We analyze a mathematical model of the dynamics of a typical transcriptionally regulated antibiotic response [3]. We identify strategies of gene regulation that optimize this response for different types of selective pressures, which we define as a set of costs associated with the drug, enzyme, and repressor concentrations during the response.

B. Experimental evolution shows loss of regulation in fast-changing environments

Using continuous cultures of tetracycline resistant *E. coli*, we perform experimental evolution to identify mutations that adapt drug responses to different dynamical regimens of drug administration. When cultures are evolved under gradually increasing drug concentrations, we predominantly observe mutations affecting alternative mechanisms of resistance. When the cultures are instead periodically exposed to large doses of antibiotics, a regimen we expected to favor the maintenance of regulation of resistance, we observe dominance of transposon insertions resulting in loss of regulation. We apply our model to show that sudden exposure to large drug concentrations can overwhelm regulated responses, which cannot induce resistance fast enough, resulting in fitness advantage for constitutive expression of resistance. These results help explain the frequent loss of regulation of antibiotic resistance by opportunistic pathogens evolving in clinical environments.

III. Conclusion

A quantitative description of how microbial populations adapt the regulation of cell responses to complex dynamical environments is crucial to understand other community-level behaviors such as pathogenesis and biofilms, as well as generating synthetic systems for biotechnology applications.

References

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