Synthetic 5' UTRs can either up- or down-regulate expression upon RBP binding

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***Short Abstract* — The construction of complex gene regulatory networks requires both inhibitory and up-regulatory modules. However, the vast majority of RNA-based regulatory "parts" are inhibitory. Using a synthetic biology approach combined with SHAPE-Seq, we explored the regulatory effect of RBP-RNA interactions in bacterial 5'-UTRs. By positioning a library of RNA hairpins upstream of a reporter gene and co-expressing them with the matching RBP, we observed a set of regulatory responses, including translational stimulation, translational repression, and cooperative behavior. Our combined approach revealed three distinct states *in-vivo*: in the absence of RBPs, the RNA molecules can be found either in a molten state that is amenable to translation, or a structured phase that inhibits translation. In the presence of RBPs, the RNA molecules are in a semi-structured phase with partial translational capacity. Our work provides new insight into RBP-based regulation and a blueprint for designing complete gene regulatory circuits at the post-transcriptional level.**

***Keywords* — RBP-RNA interactions, translation stimulation, phase transitions, SHAPE-seq**

I. PURPOSE

Regulatory networks require both inhibitory and up-regulatory modules. The vast majority of known RBP regulatory mechanisms are inhibitory. Several studies have attempted to engineer activation modules utilizing RNA-RBP interactions, based on different mechanisms. However, despite these notable efforts, RBP-based translational stimulation is still difficult to design in most organisms.

In this study, we employ a synthetic biology reporter assay and *in vivo* SHAPE-Seq approach to study the regulatory effect controlled by an RBP bound to a hairpin within the 5' UTR of bacterial mRNA, following a design introduced by[1]. We found a translationally-active and weakly-structured 5' UTR state, a translationally-inactive and highly-structured 5' UTR state, and an RBP-bound state with partial translation capacity. As a result, the same RBP (coat proteins from the bacteriophages GA, MS2, PP7, and Qβ) can either up-regulate or down-regulate expression, depending on 5' UTR sequence context. This finding deviates from the classic two-state regulatory model, which is often used as a theoretical basis for describing transcriptional and post-transcriptional regulation[2]. In a two-state model, a substrate can either be bound or not bound by a ligand, leading to either an active or inactive regulatory state. This implies that in the two-state scenario, a bound protein cannot both be an "activator" and a "repressor" without an additional interaction or constraint which alters the system.

The appearance of two distinct mRNA states in the non-induced case *in vivo,* suggests that *in vivo* the mRNA molecules can fold into one of two distinct phases: a molten phase that is amenable to translation, and a structured phase that inhibits translation. A previous theoretical study by Schwab and Bruinsma (SB)[3] showed that a first-order phase transition separating a molten and a structured phase for mRNA can occur, if a strong attractive interaction between the non-base-paired segments of the molecule exists within the system. Such an interaction destabilizes the base-pairing of branched structures, and if sufficiently strong leads to complete melting of the molecule into a non-structured form. It is possible that such attractive interaction between non-base-paired segments is mediated by the ribosome, which is known to destabilize base-paired structures during translation.

II. CONCLUSION

Our work[4] presents an important step in understanding and engineering post-transcriptional regulatory networks. The synthetic regulatory modules can be viewed as a new class of "protein-sensing-riboswitches", which, given the hypothesized phase-based characterization, may ultimately have a wide utility in gene regulatory applications. Therefore our findings imply that RNA-RBP interactions can provide a platform for constructing gene regulatory networks that are based on translational, rather than transcriptional, regulation.

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Verticalization of bacterial biofilms

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***Abstract*— Biofilms are communities of bacteria adhered to surfaces. Recently, biofilms of rod-shaped bacteria were observed at single-cell resolution and shown to develop from a disordered, two-dimensional layer of founder cells into a three-dimensional structure with a vertically aligned core. Here, we elucidate the physical mechanism underpinning this transition using a combination of agent-based and continuum modelling.**

***Index Terms*— bacterial biofilms, active matter, morphogenesis, biomechanics, development, nematic order, front propagation.**

I. Purpose

Biofilms are groups of bacteria that adhere to and grow on surfaces [1]. Understanding how biofilms form promises industrial and pharmaceutical applications [1], [2]. Recent advances in imaging technology allowed entire *V. cholerae* biofilms to be observed at single-cell resolution in real time, revealing a growth program consisting of several architectural transitions [3], [4]. These observations showed that cells are not arranged randomly within a colony, but instead grow from a two-dimensional layer of founder cells into a three-dimensional structure with a vertically-aligned core. Although cell-to-surface adhesion proteins were identified as necessary for the emergence of this vertical ordering [4], the nature of this physical process has remained unclear. In particular, how can initially prone cells overcome surface adhesion to reorient vertically, and what determines the morphology of the resulting colony?

II. Agent-based model

We show that the observed structural and dynamical features of growing biofilms can be reproduced by a simple, agent-based model. Our model treats individual cells as growing and dividing rods with cell-to-cell and cell-to-surface interactions, and thus serves as a minimal model for a wide range of biofilm-forming bacterial species. By examining individual cell verticalization events, we show that reorientation is driven by localized mechanical instabilities occurring in regions of surface cells subject to high in-plane compression. These threshold instabilities explain the tendency of surface-adhered cells to reorient rapidly following cell division.

III. Two-fluid continuum model

We incorporate these verticalization instabilities into a two-fluid continuum model. In this limit, the interplay between cell growth and cell verticalization gives rise to an exotic mechanical state in which the effective surface pressure becomes constant throughout the growing core of the biofilm surface layer. This dynamical isobaricity determines the expansion speed of a biofilm cluster and thereby governs how cells access the third dimension. In particular, theory predicts that a longer average cell length yields more rapidly expanding, flatter biofilms. We verify these predictions in experiments in which we use chemicals that alter cell length.

IV. Conclusion

Taken together, our agent-based and continuum models elucidate how the mechanical and geometrical features of individual cells control the emergent features of the biofilm, which are relevant to the survival of the collective. Thus, we expect that individual cell parameters have evolved in response to selective pressures on global biofilm morphology. Since optimal morphology may be condition-dependent, cells may also have evolved adaptive strategies that alter biofilm architecture, which could be investigated experimentally by screening for environmental influences on cell size, shape and surface adhesion.

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Stochasticity, immortality, and mortality in *E. coli*

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***Short Abstract* — Here we show that the bacterium *Escherichia coli* exhibits both lineage mortality and immortality. The outcome depends on a whether a balance is achieved between damage accumulation and the asymmetric allocation of damage from mother to daughters. At low damage rates, both old and new daughters, which are allocated respectively more and less damage, generated immortal lineages that achieved stable growth rate equilibria. At high rates, mortality ensued because while the new daughter lineage persisted, the old daughter lineage stopped dividing. The stoppage was found to result from an increase in the stochasticity of cell growth.**

***Keywords* — Cell aging, damage, mortality, immortality, asymmetry, and cell lineages.**

I. Introduction

Although cellular aging, the progressive decline of function with time, often results in the mortality of the lineage, some cells are able to sustain long-lasting immortality, as exemplified by stem cells. Following the report that mother cells of the bacterium *E. coli* partitioned damage asymmetrically by allocating more to one of her two daughters [1-3], we used predictive computational modeling [4] to ascertain whether the outcomes of both mortality and immortality could both be achieved in bacteria.

Our modeling analyses reveal that asymmetric partitioning creates different equilibria for the growth rates of old and new daughters, which receive respectively more and less damage from the mother. The equilibria result from a balance between the amount of damage a cell receives at birth, the amount it acquires while growing up to division, and the 50% dilution of the damage imposed by the division. Because the old daughter is given more damage, its lineage equilibrates at a lower value. However, because both lineages persist, the cells are immortal. If the damage rate is elevated above a threshold, the equilibrium value for the old daughters decreases to zero. The bacterial population is now mortal because all mothers produce old daughters that eventually stop dividing. Nonetheless, the population survives because the non-zero equilibrium of the new daughters allows them to persist, much like a lineage of stem cells. If the damage rate is elevated to an extreme, both equilibria become zero and the population dies.

II. Results and Discussion

To begin a test of the predictions of our model, we analyzed the growth rate of single *E. coli* cells by time-lapse microscopy in microfluidic devices with different levels and sources (phototoxicity, heat and streptomycin) of damage [6]. We have previously confirmed by using microfluidic devices of different sizes and designs that our observed differences in growth rates are not artifacts of starvation [7].

Here we show that the main predictions of our computational model were supported by our *E. coli* study. We observed equilibria for both the old and new daughters. The equilibria were also observed in mutants lacking repair chaperones, suggesting that intact repair was not required for immortality. Increasing damage rates above a threshold triggered the death of the old daughter lineages. However, an unexpected outcome was that the death was caused more by an increase in the variance (stochasticity) of growth rates for individual cells rather than by a drop of their mean growth rate to a value of zero.

Asymmetric partitioning is beneficial to bacteria because it allows cells to survive damage levels that kill symmetrical cells [4]. The asymmetry may not be unique to bacteria, as similar processes are being reported for stem cells [7-8]

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What a Lysis Pattern Tells about Phage-Bacteria Co-Propagation

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***Short Abstract* — Coexistence of phages and host bacteria is important for maintaining microbial communities. In a migrating microbial community, coexistence of bacteria and phages implies their co-propagation in space. Here we combine mathematical modeling and an experimental approach to explore how phages and motile host bacteria coexist and co-propagate, using a system of lysis pattern formation. We found that phage-bacteria co-propagation is insensitive to extrinsic factors, such as nutrient level, but critically depends on a balance between intrinsic physiology of the phages and bacteria. Highly similar phenomena indicate this physiological balance in a range of bacteria and cognate phages.**

***Keywords* — phage-bacteria interactions, co-propagation, coexistence, spatial ecodynamics**

I. Introduction

Viruses that specifically target bacteria, bacteriophages or phages, are critical components of the microbial world [1]. As obligate parasites, phages must coexist with their host bacteria at the population level.

Natural microbial communities are usually not steady in space and time. Motile bacteria can migrate towards nutrient-enriched areas via chemotaxis [2, 3], and phages can ride along the infected bacteria [4]. In a migrating microbial community, the problem of phage-bacteria coexistence becomes a problem of phage-bacteria co-propagation, i.e., the viruses and hosts need to maintain coexistence during the dynamic migration process.

II. Results and Conclusion

In this work we explored the co-propagation of phages and bacteria using a simple experimental design: phages and bacteria were coinoculated with a certain distance apart in a soft agar nutrient medium that allow the bacteria to swim. The bacterial colony initially expanded in a circle until it encountered the phage inoculum. This experimental setup mirrors realistic scenarios in which a migrating bacterial community encounters phages. After several hours of incubation in the experiment, a highly reproducible circular wedge-shaped lysis pattern emerged.

The observed phage lysis pattern cannot be explained by any previous mathematical models on formation of phage lysis patterns, which inevitably predict a circular pattern like the phage plaques formed on top agar overlays. In this work, we constructed a new mathematical model for the spatial ecodynamics of phages and bacteria, which successfully captured and explained the observed lysis pattern. The model revealed that local depletion of nutrients by bacteria is key to preserving the geometric asymmetry during formation of the lysis pattern and preventing it from becoming circular.

The model showed that the straight radial boundaries of the circular wedge pattern presents a tell-tale sign that the phages and bacteria are able to coexist and co-propagate for extended period of time. According to the model, formation of the wedge pattern is insensitive to extrinsic factors, such as the overall nutrient level and initial numbers of phages and bacteria in the inocula; these results were experimentally verified. Furthermore, the model predicted a critical dependence of the wedge pattern, particularly the straight radial boundaries, on a balance between intrinsic physiological properties of bacteria and phages associated with their proliferation and spatial spreading, e.g., bacterial division rate, motility, chemotaxis, and phage adsorption rate. Taken together, our findings suggest that extended co-propagation of phages and bacteria relies on their physiological balance.

Interestingly, similar circular wedge lysis patterns with straight radial boundaries were observed in phylogenetically distant bacterial species paired with their cognate phages. This observation implies that natural pairs of phages and bacteria generally satisfy the physiological balance, likely as a result of their coevolution under the selective pressure of maintaining coexistence and co-propagation in a migrating microbial community.

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A compact synthetic pathway rewires cancer signaling to therapeutic effector release

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***Short Abstract* — An important goal in synthetic biology is to engineer biochemical pathways to address unsolved biomedical problems: cancers. Here, we describe a method named Rewiring of Aberrant Signaling to Effector Release (RASER), where oncogenic ErbB-receptor activity, instead of being blocked as in existing treatments, is co-opted to trigger therapeutic programs. RASER integrates oncogenic ErbB-activity and links to desired outputs. A complete mathematical model of RASER and modularity in design enable rational optimization and response programming. RASER induces apoptosis and CRISPR/Cas9-mediated endogenous gene transcription specifically in ErbB-hyperactive cancer cells. RASER thus introduces a new concept for oncogene-specific cancer detection and treatment.**

***Keywords* — Synthetic Biology; Cancer; Cell signaling; Protein engineering; Signal modeling; Site-specific protease; Cancer therapy**

I. Introduction

THE specific identification and ablation of cancer cells is a long-standing problem in medicine that has not been fully solved. Cancer cells differ from normal cells in their ability to proliferate and survive in an uncontrolled manner, a consequence of mutations that drive the constitutive activation of intracellular signaling pathways. Signals commonly activated in cancer have been targeted for suppression by drugs, but these drugs are limited by toxicity from inhibiting normal signaling as well. We considered a fundamentally different approach to cancer treatment in which oncogenic signals are detected and then, instead of being suppressed, are co-opted to trigger therapeutic programs. Here, we describe Rewiring Aberrant Signaling to Effector Release (RASER) in which a synthetic signaling pathway detects an oncogenic signal with high specificity, and then rewires it to a variety of customizable responses [1].

II. Results

We chose constitutive activation of ErbB proteins (EGFR and HER2), as a target for detection and rewiring of oncogenic states. We designed a synthetic two-component system to integrate ErbB output over time and discriminate between constitutive and normal signaling. Modularity was introduced to enable a variety of outputs, and a complete mathematical model of RASER was developed to enable rational optimization. The resulting ErbB-RASER system detected constitutive ErbB activity in a variety of ErbB-driven cancer cells, with responsivity exceeding that of endogenous pathways downstream of ErbB. ErbB-RASER was successfully programmed to induce apoptosis and CRISPR/Cas9-mediated transcription of endogenous genes such as cytokines specifically in ErbB-hyperactive cancer cells. Delivery of apoptotic RASER by adeno-associated virus selectively ablated ErbB-hyperactive cancer cells while sparing ErbB-normal cells.

III. Conclusion

RASER introduces a new concept for cancer detection and treatment, in which specific oncogenic signals are detected in cancer cells and then used to trigger a programmable therapeutic response. Our study demonstrates that (i) synthetic signaling pathways based on first principles can detect an oncogenic protein activity with specificity matching or exceeding natural signaling pathways ; (ii) mathematical models can predict the compact synthetic signaling systems ; (iii) finally, the selectivity and potency of ErbB-RASER to ablate ErbB-hyperactive tumor cells suggests therapeutic potential. Further generalization of RASER to other inputs and outputs could enable the development of a panel of active biological therapies targeted to specific cancerous states.

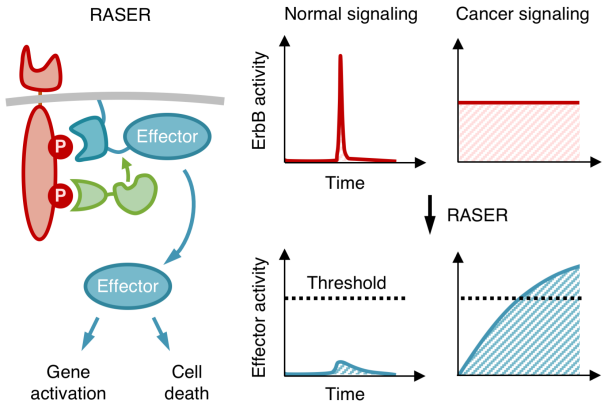

Figure 1 Rewiring Aberrant Signaling to Effector Release (RASER) in cancer cells. Left, in response to ErbB (EGFR or HER2) activity, RASER proteins (green and blue) release a programmable effector to carry out therapeutic responses. Right, RASER transforms normal signaling, which is transient, to low and transient accumulation of effector. In contrast, with constitutive oncogenic signaling, effector accumulates until a therapeutic threshold is reached.

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Stochastic Activity of a Bacterial Sensory Network

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Abstract— We combined single-cell FRET measurements with analysis based on fluctuation-dissipation relations to directly measure and dissect a posttranslational signaling noise in the chemotactic sensory pathway of Escherichia coli, predicted to enhance environment exploration. Interactions between the clustered pathway receptors amplified two noise sources of comparable amplitudes, energy-consuming stochastic methylations and, surprisingly, thermal noise, to produce the unexpectedly large pathway activity fluctuations we observed. The high response sensitivity of this sensory pathway therefore also increases its susceptibility to thermal and out-of- equilibrium noises. Our study glanced at the richness of the little studied stochasticity in posttranslational pathway behaviors, beyond gene expression.

Index Terms— stochasticity, motility, chemotaxis, FRET, Escherichia coli, posttranslational noise

I. Introduction

Although cellular noise relating to the variability in expression of genes or proteins is often well characterized, little is directly known of stochastic processes arising in signaling networks at the posttranslational level. In the chemotaxis signaling pathway of E. coli, such noise has been predicted based on analyses of cell motility [1]. This bacterium swims in a random walk of straight, flagella-propelled, runs, interrupted by short random reorientations, or tumbles. The chemotaxis pathway is in charge of navigating in environmental gradients [2]: Changes in conditions are monitored and amplified by clustered transmembrane receptors and rapidly (< 0.1 s) transduced via a phosphorylation signal to the flagellar motors to prolong runs when conditions get better. Two low-aboundance adaptation enzymes slowly (few seconds) actuate a receptor-methylation-based memory to counterbalance previous changes in phosphorylation activity, allowing the bacterium to always compare its current situation with the recent past.

In homogeneous environments, the duration of the runs was shown to fluctuate strongly over a 30 s time scale [1]. This behavior was previously attributed to putative small fluctuations of the phosphorylation activity of the pathway due to stochastic methylation events, and amplified at the level of the flagellar motors [3].

The population-averaged pathway activity has long been measured by an assay based on the Förster resonance energy transfer between two fluorophores in close proximity, which tag two molecules that interact in an activity-dependent fashion. We improved it to measure pathway activity at the single cell level and investigate its temporal stochasticity [4].

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II. Results

The amplitude of the temporal activity fluctuations we measured was similar to the maximal response of the pathway itself, i.e. ten times larger than initially expected, suggesting that motor-level amplification was not necessary. Their time scale matched that of the run-duration fluctuations (30 s). Moreover, contrary to previous hypotheses, mutants lacking the adaptation enzymes also showed fluctuations, of a similar amplitude but on a longer time scale (~ 100 s). Finally, mutants disrupting receptor cooperativity, but not signaling, produced no activity fluctuations. These results clearly indicated that two noise sources, one of which was the stochastic methylation events as expected, were strongly amplified by the cooperative array of receptors.

We showed that the unexpectedly large amplitude of the fluctuations came from the receptor cooperativity units being larger than previously assumed[5]. We then compared activity fluctuations with the pathway response to a small stimulation, in the adaptation-less mutant. Their ratio being close to the room temperature indicated, according to the fluctuation-dissipation theorem, that thermal noise was the second source of noise, amplified by the receptor cluster and distorted by its slow rearrengements.

III. Conclusion

These results highlight the importance of energy-consuming but also passive thermal noises within signaling sensory pathways, even at posttranslational level, opening questions on the frequency of, and bacterial adaptations to, this type of stochasticity.

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Programmable Protein Circuits in Living Cells

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Synthetic protein-level circuits could enable new cellular behaviors, and their design would be facilitated by a composable system in which individual components can regulate one another to create a variety of architectures. We engineered viral proteases into such composable components, and implemented diverse functions in mammalian cells[1]. Furthermore, we designed a circuit that induces cell death in response to upstream activators of the Ras oncogene. Because protein circuits can perform complex functions yet be encoded as single transcripts and delivered without genomic integration, they offer a scalable platform to facilitate protein circuit engineering for biotechnological applications.

I. INTRODUCTION

MAMMALIAN synthetic biology aspires to emulate natural pathways with synthetic biomolecular circuits and program new cellular functions, and it holds great promise for basic research and biomedicine[2-3]. Synthetic circuits have largely relied on gene regulation and especially transcriptional control[2-3]. However, many natural pathways operate post-translationally[4], and synthetic protein circuits could offer benefits such as faster operation, direct coupling to more signaling pathways, and compact encoding on a single transcript. What has been lacking for protein circuits is "composability"[5]. Composable circuit components share the same form of inputs/outputs (e.g., transistors), and can be connected differently for various tasks.

Viral proteases provide a promising basis for such a system. Many of them exhibit strong specificity for short cognate target sites, which can be recognized and cleaved in a variety of protein contexts[6-8]. Viral proteases have been used in conjunction with degrons to control the stability of other proteins in a modular fashion[9-11], and have been coupled to transcription factors. The extensive diversity of viruses provides multiple proteases that cleave with distinct site specificities, potentially enabling scalable and orthogonal regulation[12]. Here we show that viral proteases can be engineered to regulate one another, addressing the key challenge of composability, and enabling the design of diverse protein-level circuits.

II. RESULTS

We first established fluorescent reporters for protease activity, tagged with degrons that can be removed/activated by proteases[1]. Using these reporters, we validated a set of three orthogonal proteases[1]. Finally and most importantly, for composable protease-protease regulation, we engineered viral proteases to repress each other through docking-enhanced direct cleavage[1].

With such composable components, we successfully demonstrated diverse computational functions, including logic gates, bandpass filter, and adaptive pulsing, in human embryonic kidney (HEK) cells[1]. These new functions, many for the first time in any mammalian system, represent basic signal processing modules prevalent in biology and useful for engineering more complex synthetic circuits. Eyeing direct therapeutic applications, we also implemented a prototypic circuit to induce cell death in response to activators of the "undruggable" oncogene Ras[13], and showed that selectivity could be enhanced through rational circuit engineering[1].

III. CONCLUSION AND FUTURE DIRECTIONS

Our proof-of-principle work illustrates several features of protein circuits: recurring motifs (e.g., threshold setting) applicable in diverse contexts, compact encoding on single transcripts, and fast design-build-test cycles. They lay the foundation for a general-purpose platform for the composable construction of protein circuits.

The specific "Ras killer" circuits has clear medical implications, and will be further optimized in cancer cell lines and animals models. Viral vectors for delivering such circuits into patient cells will also be explored.

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Bayesian analysis as a tool for mechanistic inference in cell signaling systems

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Short Abstract – We present two applications of Bayesian Parameter Estimation to reduce systems biology models. First, we learn reduced models from data using a combination of Parallel Tempering (PT), an accelerated sampling method, and Lasso, a sparsity-promoting penalty common in Machine Learning. Next, we use a mixed effects approach with a reduced model of NFκB signaling to learn differences in protein expression that explain observed single-cell variability. We conclude that this two-tiered approach can be used to improve our understanding of core signaling mechanisms and the mechanistic basis of heterogeneity, which in turn will enable development of strategies to manipulate single-cell decisions.

I. Introduction

Computational models play an important role in studying biological systems.[1] Although detailed models are useful for summarizing knowledge, they have a large number of unknown parameters, making it difficult to estimate and analyze the high dimensional parameter spaces that fit the data. Reduced models can be simpler to interpret and communicate and can provide simplified mechanistic explanations of experimental data.

We consider two types of model reduction and provide Bayesian solutions to both: (1) reduction of the architecture using PT with Lasso and (2) reduction of the parameter space required to explain heterogeneity in single-cell responses. Heterogeneity is common in cell signaling,[2] and mechanistic explanations of why cells respond to the same stimulus differently may help design more effective treatments. We hypothesize that different cell behaviors can be explained by cell-specific parameters, and apply a Bayesian mixed-effects modeling approach to NFκB signaling data to find reduced parameter spaces that capture single-cell heterogeneity.

II. Methods

Inferring model structure: PT is an accelerated sampling method[3] that is more efficient than the conventional Metropolis-Hastings algorithm for Bayesian parameter estimation in systems biology.[4] We combined PT with Lasso[5] to find minimal sets of non-zero parameters that fit the data. The zero parameters correspond to parts of the model that are not needed to fit the data. We extended this approach to select over modules within reaction-networks instead of individual reactions.

Population modeling: To explain heterogeneous single-cell NFκB responses we used a Bayesian variant of mixed effects modeling[6], an approach that jointly infers both individual and common parameters from individual responses to a common stimulus.

III. Results

1) *PT with lasso can infer motifs from reaction-networks.* For a number of common motifs (e.g. a perfectly adapting dose-response), we found that PT with lasso was able to recover the motif from synthetic data starting from a prior network of greater complexity.

2) *A20 feedback is not required to fit NFκB responses to a short pulse of TNF.* We divided the NFκB signaling network into biologically-motivated modules in order to determine which were required to fit single-cell NFκB data. Our extended Lasso approach removed the A20 module, demonstrating that negative regulation by A20 was not necessary to fit representative data.

3) *Varying protein expression in a reduced model of NFκB signaling captures observed heterogeneity.* Using our population modeling approach, we found that changes in protein expression alone could account for variability in single-cell responses to a TNF pulse. We also found that dose response properties such as bandwidth and EC50 can be explained by differences in protein expression.

IV. Concluding remarks

We have shown that PT with Lasso can infer reduced models from both synthetic and real data, and we have used our reduced model of NFκB signaling to make mechanistic inferences about the system based on single-cell responses to short TNF pulses. In one analysis we found that the A20 feedback loop is not necessary to fit representative single-cell responses. One caveat from this analysis was that it did not constrain the behavior of other biochemical species, and we are currently checking whether all parameters of the reduced models are biologically feasible. In another analysis we found that differences in protein expression alone could account for the observed variability of responses and further that distinct clusters of responses could be mapped back to specific regions of the protein expression space. We are currently extending our analyses to determine whether these findings will hold under other stimulation conditions.

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Waddington's Landscape of Cell Aging

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***Short Abstract* — Using microfluidic and time-lapse imaging technologies, we investigate single-cell aging dynamics of yeast *S. cerevisiae*. We found two modes of aging processes in single aging cells with distinct phenotypic changes and death forms and identified molecular pathways that drive the two aging processes. Computational modeling revealed that the two pathways interact dynamically to enable an early-life switch, governing aging fate decision. Model-guided perturbations can reshape the fate decision landscape and dramatically extend the lifespan.**

***Keywords* — Cellular aging, single-cell analysis, systems biology, computational modeling, fate decision, microfluidics**

I. Introduction

Cellular aging is a complex process that involves many interwoven molecular processes [1,2]. It is also a dynamic and stochastic process wherein genetically identical cells have various intrinsic causes and widely different rates of aging. As a result, traditional population-based studies focusing on static gene mutations are insufficient to unravel complexities that underlie the aging process, hindering the mechanistic understanding of cellular aging. Studies in model organisms have identified many individual genes and factors that have profound effects on lifespan [3]. However, how these genes and factors interact dynamically and function collectively to drive the aging process remains unclear.

II. Results

To meet these challenges, we exploited newly-developed microfluidic and time-lapse imaging technologies to investigate single-cell aging dynamics throughout the replicative lifespans of *S. cerevisiae*. We found that isogenic cells diverge towards two aging paths, with distinct phenotypic changes and death forms. We further identified specific molecular pathways driving each aging fate and revealed that these pathways interact and operate dynamically to enable an early-life switch that governs the aging fate decision and the progression towards death. These two modes of single-cell aging processes can be quantitatively depicted as the diverged progression towards two stable points in the aging landscape. Model-guided genetic perturbations of the molecular circuit reshape the landscape and dramatically extend the lifespan.

III. Conclusion

Our work uncovers the interconnected molecular pathways that drives the aging process and opens up the possibility of designing interventions that simultaneously target multiple network nodes, instead of single genes, to more effectively extend the healthspan. Furthermore, our findings demonstrate that cellular aging is a tightly-regulated fate decision process rather than a consequence of passive damage accumulation. Given that the molecular network underlying the fate determination is deeply conserved, aging-driven cell differentiation might represent a general scheme in many organisms.

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Synchronizing mouse presomitic mesoderm cells *Ex vivo* and *in silico* approaches

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Somitogenesis is a time and space periodic process of the vertebrate body axis segmentation which requires coordinated genetic oscillations. Numerous studies show that the Notch signaling pathway is involved in synchronizing the oscillations on a tissue level. However the coupling rules remain unclear. Many studies in our field used the phase-difference coupling model as a standard model to explain synchronization during somitogenesis. We use live imaging with a fluorescent reporter of Notch pathways combined with phase extraction methods to directly compare the theoretical predictions to experimental outcomes. Our results challenge the standard theory to describe the rules of coupling of segmentation clock.

***Keywords* — phase synchronization, oscillations, phase-difference coupling model, pulse coupling model , self-organization , presomitic mesoderm cells, somitogenesis.**

I. Biological context

During mouse embryogenesis, presomitic mesoderm (PSM) elongates posteriorly, while its anterior part undergoes a periodic segmentation forming one pair of somites every 120min. Periodical waves of Notch, FGF and Wnt signaling activity traverse the tissue from the posterior end to the anterior. This pattern of gene expression waves is achieved by coordination of each PSM cell's activity, each cells functioning as an autonomous genetic oscillators. Previously, using real time imaging of a dynamic Notch signaling reporter, LuVelu (Lfng-Venus-Lfng) in mouse (1), our group observed that genetic oscillators from PSM cells display periodic wave in a two-dimensional culture system(2).

Subsequently, a novel in vitro approach has been established to address the question of the origin of spatiotemporal wave patterns (3). Surprisingly, after randomization, PSM cells can self-organize into several miniature emergent PSM structures (ePSM). Randomized PSM cells with mixed frequencies and phases are able to synchronize de novo, leading to the formation of ordered patterns oscillating in sync. From numerous perturbations studies, it is well know that the Notch signalling pathway is involved in synchronizing PSM oscillations on a tissue level but the rules of coupling remain elusive.

II. Theoretical and experimental approaches

To describe how the coupling is achieved, theoretical physics propose two distinct classes of models: phase-difference coupling models and pulse coupling models. To explain synchronization in PSM cells, main studies in our field used the phase difference coupling framework and indeed these models (4, 5) can recapitulate several aspects of PSM oscillations. However, a central prediction of phase-difference framework *per se* is still unclear. In addition, numerical simulations performed in the lab show that phase-difference and pulsed coupled synchronization models make distinct predictions regarding collective phase determination. This highlights the importance to find a new experimental study, we have established a new experimental setup which allows us to quantitatively test the predictions made in phase-difference and pulsed coupling models.

III. Conclusion

Our results are not in agreement with the current segmentation clock synchronization models. In contrast, our findings are compatible with the synchronization mechanism based on pulsed coupled oscillators. We are currently further challenging these conclusions using both theoretical and experimental strategies. In summary, this study is calling in question our fundamental understanding of coupling rules governing the cell synchronization during segmentation clock.

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Microtubules Negatively Regulate Insulin Secretion in Pancreatic Beta Cells.

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Short Abstract — Two key prerequisites for glucose stimulated insulin secretion (GSIS) in Beta cells are the proximity of insulin granules to the plasma membrane and their anchoring or docking to the plasma membrane (PM). Here we use imaging and computational modeling to demonstrate that microtubule (MT) motor mediated transport dynamics have a critical role in regulating both factors. First, the MT network preferentially withdraws insulin granules from the PM, contrary to conventional wisdom. Second, the binding and transport of insulin granules by peripheral MT motors prevents their stable anchoring to the PM. The MT cytoskeleton thus negatively regulates GSIS by both limiting the amount of insulin proximal to the PM and preventing/breaking interactions between the PM and the remaining nearby insulin.

Keywords **— Glucose stimulated insulin secretion; computational modeling; membrane docking; anomalous diffusion**

INTRODUCTION

Dysregulated Glucose Stimulated Insulin Secretion (GSIS) results in diabetes, a disease that afflicts ~9% of the US population. Thus, elucidating how GSIS is regulated is of fundamental importance in understanding metabolic homeostasis. It has long been known that GSIS is systemically regulated. But that regulation must be implemented at the level of individual Pancreatic islet beta cells, the insulin factories in the body.

The major stimulant for insulin secretion is high glucose, whose entry into and subsequent metabolism in beta cells triggers secretion. Interestingly, <1% of a cells stored insulin is secreted in response to stimulation, suggesting the presence of tight regulation at the level of individual cells. A main determinant of insulin secretion dosage at given stimuli is the number of readily releasable insulin vesicles, namely those that are biochemically capable of anchoring at the secretion sites and close enough to the plasma membrane to do so. Here we demonstrate that cells use the cytoskeleton to regulate this readily releasable pool (RRP).

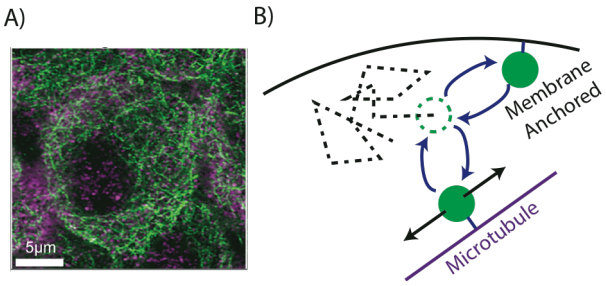

Figure 1: A) Image of Beta cells depicting MTs (green) and insulin granules (purple). B) Illustration of computational model of insulin granule dynamics.

Results

It has long been though that the cytoskeleton regulates insulin dynamics in Beta cells. Interestingly however, prior imaging of intact islets indicate that MT network these resembles an undirected random mesh and that granule motions are random and sub-diffusive, suggesting that directed transport is not the main function of MTs in these cells. How then do MTs influence GSIS?

To address this question, we first used super-resolution microscopy to quantify the directionality of the MT network. Results demonstrate that while away from the cell border the MT network resembles a random interlocked mesh, within a narrow peripheral region it exhibits significant co-alignment with the cell border. Computational modeling results demonstrate two consequences of this structure. First, MT bound and unbound insulin form counter propagating gradients with granules primarily being delivered to the PM in a MT independent fashion and withdrawn from the periphery by the MT network. Second, motor driven traction forces on the parallel array of MTs prevent stable granule anchoring to the membrane. Thus MTs serve to both remove granules from the peripheral regions of the cell and prevent stable anchoring of those that remain to the PM, both of which would negatively regulate GSIS.

CONCLUSION

Our work sheds new light on how this GSIS regulation is accomplished at the level of individual cells. Specifically, the microtubule (MT) cytoskeleton has a previously undiscovered but critical role in regulating the exocytosis of insulin granules (GSIS) these cells. These results may point to new strategies and therapeutic targets for regulating GSIS. They also elucidate two novel mechanisms by which exocytosis can be regulated by the cytoskeleton.

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Faster Growth Reduces the Sensitivity of Gene Circuits to Environmental Signals

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***Abstract*— Single-celled organisms adapt to changing environments by gene regulatory switches that sense chemical cues and induce specific genes when inducing signals are over a critical threshold. We measure the switching dynamics of the *lac* operon in single cells, when nutrients change from glucose to lactose, and find that cells become ultra-sensitive to lactose when they transiently stop growing at the transition. Mathematical modeling shows that the sensitivity of regulatory switches to external signals generally decreases with growth rate. This growth-coupled sensitivity constitutes a mechanism through which cells become highly sensitive to alternative nutrients or stresses when they stop growing.**

I. Background

Among the regulatory circuitry that is employed by unicellular organisms to sense and adapt to environmental changes, one of the most commonly occurring architectures are positive feedback switches. In these regulatory switches a positive feedback between the sensing of a chemical signal and resulting changes in expression of a set of target genes lead the system to switch its gene expression state in an almost binary manner from an uninduced to an induced state at a critical concentration of the signal [1].

Although such bistability is well understood from mathematical models of positive feedback switches, very little is known about the stochastic dynamics at the single-cell level, and what variables determine if and when a single cell switches its expression state.

II. Results

We here use a combination of microfluidics, time-lapse microscopy and quantitative image analysis [2] to study in single cells the stochastic induction dynamics of the archetypical example of such a regulatory switch, the native *lac* operon of *E. coli*, when the carbon source is changed from glucose to lactose. In contrast to the case of artificial inducers which don't impact growth [3], we find that cells become ultra-sensitive to lactose when they transiently stop growing after changing nutrients. Mathematical modeling shows that, due to a natural competition between uptake of the inducer and dilution due to growth, the sensitivity of regulatory switches to their external signals generally decreases with growth rate. We show that this allows cells to implement a strategy in which the decision to use a new carbon source depends both on its concentration and on the current growth rate, and only occurs when the use of the new carbon source increases growth rate. Moreover, the way in which the global regulator CRP is known to regulate its targets is precisely matched to implement this strategy [4].

III. Conclusion

Growth-coupled sensitivity provides a general mechanism through which cells can 'mute' external signals in beneficial conditions when growth is fast, and become highly sensitive to alternative nutrients or stresses when growth is slow or arrested. We discuss how many of the known architectures of regulatory switches in bacteria appear designed to exploit growth-coupled sensitivity.

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Combinatorial logic of BMP signaling

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***Short Abstract* — Combinations of Bone Morphogenetic Proteins (BMPs) promote different cell fates, such as bone and cartilage, but it remains unclear whether these BMPs signal as combinations of redundant or unique inputs. By measuring pathway responses to all possible pairs of BMPs, we show that some, but not all, BMPs are functionally equivalent, and that BMP amino acid sequence cannot necessarily predict functional equivalence. Moreover, theory and experiments show that changes in BMP receptor expression can rationally reprogram these equivalence relationships. These results show how a cell's receptor expression selects for the relevant ligand differences when computing the overall pathway response.**

***Keywords* — Cell signaling, combinatorial inputs, BMP signaling, automated liquid handling, flow cytometry.**

I. Background

INTERCELLULAR signaling guides complex cell fates and patterns during development. Despite knowing the molecular components for most signaling pathways, the emergent logic by which these pathways encode and decode developmental information remains unclear. For example, ligands of the Bone Morphogenetic Protein (BMP) pathway are coexpressed in time and space to guide the formation of multiple tissues, including bone, heart, and brain [1]. It has long been thought that many of these ligands are redundant, as they have similar structures and sequences, and bind overlapping sets of receptors to activate the same transcription factors [2]. However, we have discovered that cells can respond differently to different ligand combinations [3], and not all BMP ligands function as perfect replacements [4]. Determining which BMP ligands are redundant or unique and in which contexts could vastly improve the therapeutic use of exogenous BMPs [5] and reveal how unique signal-processing capabilities arise from promiscuous ligand-receptor interactions.

II. Results

To probe the range of responses to combinations of BMPs, we developed a high-throughput assay for measuring responses to all possible pairs of BMP ligands. In the assay, a robotic liquid handler generates all pairwise combinations, and BMP pathway activation is read out by flow cytometry.

A. BMP ligands fall into distinct equivalence classes.

Functionally equivalent BMP ligands should activate the BMP pathway to the same extent both when signaling individually and when in combination with any other ligand. By comparing profiles of pairwise responses, we show that the ten BMP ligands we studied fall into five distinct classes, and that similarity at the level of amino acid sequence cannot predict these functional classes.

B. Equivalence classes change in a new cell line.

If ligand equivalence is a fixed property of each ligand, equivalence relationships should be preserved in a new cell line. However, when we repeated the pairwise screen and analysis in a new cell line, we observed a new set of equivalence relationships, indicating functional equivalence depends on cell context.

C. Changes in receptor expression can rationally perturb equivalence classes.

Our mathematical model of BMP signaling [3] indicates that changing the expression level and identity of BMP receptors can alter the functional equivalence of the BMP ligands. In particular, ligands signal more equivalently in regimes where each ligand has access to at least one high-affinity, active receptor. By overexpressing known high-affinity receptors, we rationally increased the functional equivalence of specific BMP ligands.

III. Conclusion

Our data suggest that the equivalence of different signaling proteins in combinations is not fixed. Rather, receiving cells, via their receptor profile, select for the relevant differences between signaling proteins. Moreover, cells could alter receptor expression to modulate their sensitivity to differentiation signals. Finally, the exact map of equivalence relationships in multiple contexts may provide a more meaningful narrative for pathway evolution, as it may describe how the pathway's signal-processing capability changes as additional components are added.

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An Accessible Microfluidic Platform to Probe the Capabilities of Single Cells

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***Short Abstract* — The microenvironment of a cell in a living system is dynamic. When exposed to different extracellular cues, such as changes in the concentration of inflammatory cytokines, cells activate signal transduction networks that mediate fate decisions. Exploring cellular responses to a broad set of time-varying microenvironments is essential to understand the information-transmission capabilities of signaling networks, and how cell fate decisions are impacted by the dynamic extracellular milieu. Here, we present a gravity-driven cell culture system for investigating cellular responses to dynamic stimuli. We demonstrate that the system accurately produces user-defined concentration profiles independently for one or more stimuli in the cell culture. Measurement of nuclear NF-κB mobilization in cells exposed to dynamic stimuli reveals context-dependent cellular sensitivity and uncharacterized single-cell responses distinct from persistent cytokine exposure. These hidden capabilities, uncovered through dynamic stimulation, provide opportunities to discover and manipulate signal transduction mechanisms in the context of in vivo microenvironments.**

***Keywords* — Microfluidics, laminar flow, NF-κB, Signal transduction, inflammation.**

I. Introduction

The microenvironment of a cell is constantly changing. When cells are exposed to extracellular cues, such as fluctuations in the concentration of inflammatory cytokines or drugs, they activate dynamic signal transduction pathways that govern pivotal cell fate decisions[1]. Although deregulation of these pathways contributes to human disease, most experiments characterize cells exposed to constant stimulation which contrast the transient and time-varying properties of cues in vivo.

Biomedical micro-electro-mechanical systems such as microfluidic devices broadly enable studies of cell behavior in precisely controlled microenvironments. However, current implementations to provide time-varying control of a cell culture often suffer from limited operating ranges and reliance on high-complexity devices that require specialized expertise to make and use. Because of these challenges, signal transduction networks are rarely investigated in the context of dynamic microenvironments.

II. Results

In this work We set out to address these challenges by developing a minimal dynamic stimulation system that provides time-varying control over cell culture composition in live-cell imaging experiments. The modular system consists of a gravity pump controller, which can be built from commonly available low-cost parts, to coordinate gravity-driven flow and laminar fluid streams in a cell culture device. Cell culture devices under control of the gravity pump are assembled from 3D-printed molds[2] on a standard lab benchtop, and can be interchanged to provide different functions. By automating flow rates in the cell culture device, the system provides independent control of concentration dynamics for multiple stimuli over a broad dynamic range.

We compare activation of the NF-κB signaling pathway in live cells exposed to tumor necrosis factor (TNF) either continuously or as a slow ramp in concentration. Although the characteristic response of the NF-κB pathway is pulsatile and adaptive[1,3,4], we find alternative patterns of pathway activation when cells are exposed to a TNF ramp. A significant fraction of cells shows nearly analog responses to ramp TNF stimulation, whereby NF-κB accumulates as a ramp in nuclei of single cells. We also find pathway activation is increased in response to ramp stimuli, producing a greater nuclear NF-κB response even though cells are exposed to significantly less TNF.

III. Conclusion

Our results demonstrate that dynamic stimulation can be achieved under gravity-controlled flow and can be used to reveal hidden capabilities of signal transduction networks.

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Evolutionary stable coexistence of microbes

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***Short Abstract* — Tremendous diversity has been observed in the microbe world. However, the constant pressure of evolution, why not a single optimal species emerge that finally dominates over all others? In this work, we explored this question by constructing a generalizable framework to describe the species-environment feedback in chemostat-type resource-competition models. Employing this framework, we revealed how species create their own fitness landscape by shaping their nutrient environment, which allows for non-stationary fitness landscapes and rich ecological behaviors, and is crucial for biodiversity. A non-invasible strategy corresponds to a species creating a fitness landscape that places itself at the top. Under certain conditions, more than one species is required to complete this task, which leads to evolutionarily stable coexistence.**

Keywords — Metabolic trade-offs/Coexistence/Optimal strategies/Chemostat

I. Motivation

In many ecosystems, not only does the environment influence species, but species also actively reshape their habitats[1]. Resource-competition models provide a simple context to explore the species-environment feedback. In such models, species interact only indirectly, via consumption (and sometimes production) of a shared pool of nutrients[2]. However, there are long-standing contradictions between theories of resource competition and the observed diversity in nature: First, on the basis of simple resource-competition models, it has been proven that the number of stably coexisting species cannot exceed the number of resources, leading to the so-called competitive exclusion principle [3]. Moreover, as species evolve to adapt to their environment, an ongoing threat to diversity is that mutation/selection may produce a supreme winner which allocates resources in the most efficient way and takes over the habitat. Indeed, in computational works that introduced evolution into a standard model of competition, it found the number of surviving species to be much lower than the number of nutrients [4]. Then how shall we understand the observed diversity and coexistence in the microbial world, particularly stable coexistence in the time-scale of evolution?

II. Results and future directions

In this work, we utilize and extend the graphical tools of resource-competition theory to relate and unify multiple metabolic models for microbial diversity, emphasizing the consequences of species creating their own environment. The nutrient environment created by one species though growth and consumption defines a fitness landscape that can be inviting or prohibiting to other species. For various metabolic models, we investigate how different species change the relation between metabolic strategy and growth rate. Such a non-stationary fitness landscape allows intransitivity of competitiveness, with no strict competition hierarchy and therefore no single best species. We demonstrated how such intransitivity can lead to rich ecosystem dynamics, including unlimited coexistence, oscillation, and multistability. To address evolutionary stability, we derived the general condition for optimal metabolic strategies – a set of species that construct a fitness landscape that places themselves on the top. When multiple species are indispensable in co-creating such an environment, their coexistence is evolutionarily stable.

One particular metabolic model we investigated, is the one allowing cross-feeding: dual-role metabolite appear in multi-step energy production reactions, which is detrimental to upstream reactions and can be released into the environment, but also beneficial for downstream reactions and can be utilized by other species as resources. In such model, we quantified the conditions for coexistence through cross-feeding to be evolutionarily optimal. Moreover, we found optimal coexistence through competition instead of cooperation. Such non-intuitive evolutionarily stable coexistence calls for more experimental and theoretical investigation.

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Antibiotic lethality depends directly on metabolic state

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Growth rate and metabolic state have both been separately shown to effect antibiotic efficacy[1]–[3]. However, bacterial growth inherently imposes a metabolic burden[4], which makes it challenging to determine individual contributions from each[4]. Here, we measured growth and metabolism in parallel across a broad range of coupled and uncoupled conditions to determine their relative contribution to antibiotic lethality under coupled and uncoupled conditions. We show that when growth and metabolism are uncoupled, antibiotic lethality uniformly depends on the bacterial metabolic state at the time of treatment, rather than the growth rate. We further reveal a critical metabolic threshold below which lethality is negligible. This metabolic-dependent antibiotic lethality provides a cohesive physiological basis for antibiotic-mediated cell death, unites previous literature findings, and provides a framework for future treatment design.

***Keywords* — Antibiotic mechanism, antibiotic resistance, bacterial metabolism**

I. Purpose

Growth rate and metabolic state have both been separately shown to effect antibiotic efficacy. For example, the lysis rate of β-lactam-treated cells is robustly correlated with the growth rate[5]. Likewise, antibiotic efficacy can be promoted or suppressed by exploiting the active role of metabolic dysregulation in antibiotic-mediated cell death, such as stimulating or inhibiting key aspects of cellular respiration[6]. It is likely that one or both aspects contributed to varying degrees, since growth and metabolism are inter-related; indeed, these processes are can exhibit a wide range of coupling (e.g., metabolic efficiency), which depends on a host of environmental and physiological factors[7]. Therefore, a clear understanding of the interdependence between growth and metabolism on antibiotic efficacy, and its implications, remains lacking.

II. Results

To investigate the extent to which growth and metabolism each contribute to antibiotic lethality, we used varying ratios of carbon (glucose) and nitrogen (casamino acids) concentrations to establish experimental conditions where the two readily exhibit coupling and uncoupling: intuitively, the high energetic burden of amino acid biosynthesis is alleviated by exogenous amino acid supplementation[8]. In the presence of excess glucose, this allows for the more efficient allocation of glucose-derived ATP towards biomass instead of amino acid production, which in turn increases growth without significantly altering respiration[9], [10].

Regardless of whether growth and metabolism were coupled or not, survival was uniformly and inversely correlated with metabolism for nine bactericidal antibiotics at two concentrations. However, when survival data are plotted verses growth rate, uncoupled conditions no longer mimic the coupled trends. In other words, growth rate was sufficient to predict survival only when coupled to metabolism. These results are general to Gram-positive and -negative clinically relevant strains, and additional metabolic conditions.

To test the extent to which intracellular ATP at the time of antibiotic treatment alone is sufficient to account for killing dynamics, we built a simplified mathematical model based on our prior results. Using this model, we show that antibiotic lethality as a function of metabolic state is sufficient to account for killing dynamics. Moreover, modeling elucidates key parameters that can be perturbed to predictably potentiate or protect metabolic-dependent antibiotic lethality, which we validate experimentally.

III. Conclusions

Our work provides a unified explanation of antibiotic killing by showing that lethality depends directly on the metabolic state of the cell, which in turn may or may not be correlated with growth. This highlights the subtle, yet non-trivial, delineation between first- and second-order antibiotic killing mechanisms. Understanding these effects will be key to developing novel therapeutics that more effectively target bacteria in non-permissive states, as well as enhancing the efficacy of existing antibiotics with appropriate adjuvants to push microbes over the critical metabolic threshold towards their death.

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Periodic treatments and immunity impact antimicrobial resistance evolution and spread

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***Abstract*— Antimicrobial treatments select for resistance, and once resistance becomes widespread, antimicrobials become useless. Resistance evolution within a host can be strongly affected by variations of antimicrobial concentration, and resistance spread in a host population can be impacted by host immunity. We investigated these effects using stochastic models. First, we focused on microbial populations subjected to periodic alternations of antimicrobial absence and presence. We showed that the probability of treatment success, i.e. of extinction of the microbial population, strongly depends on the period of the alternations. Second, we showed that antibody-mediated cross-linking of gut bacteria hinders the spread of antibiotic resistance.**

***Index Terms*— Drug resistance, Variable fitness landscape, Population genetics & dynamics, Immunity, Stochastic models**

I. Introduction

Understanding the evolution of resistance within a host and its spread in a population of hosts are of paramount importance in order to fight this major public health issue.

Mutations that confer antimicrobial resistance are often associated with a fitness cost, but this cost can be compensated by subsequent mutations. Without antimicrobial, the adaptive landscape of microbes involves a valley, because of the initial cost of resistance. However, this fitness valley disappears above a certain concentration of antimicrobial, as the growth of antimicrobial-sensitive microorganisms is impaired. Thus, the adaptive landscape depends drastically on whether antimicrobial is present or not. We showed that periodic antimicrobial concentration variations strongly impact resistance evolution [1] and treatment success.

Within-host immunity may interfere with resistance spread in a host population. It was recently shown that immunoglobulin A (IgA), the main effector of adaptive immunity in the gut, enchains daughter bacteria upon division. Bacteria being in clonal clusters decreases the effective genetic diversity of transmitted bacteria. We demonstrated that this effect can hinder the spread of antibiotic resistance in a population of hosts [2].

II. Questions and models

What is the impact of varying patterns of selection on antimicrobial resistance evolution? To address this question, we performed a stochastic study of de novo resistance acquisition in the presence of alternations of phases of presence and absence of antimicrobial. This situation may represent, for example, a treatment where the concentration within the patient falls under the Minimum Inhibitory Concentration (MIC) between drug intakes [4]. We considered homogeneous microbial populations described by birth-death processes, both with a fixed population size [1] and with a variable population size allowing extinctions.

How does gut immunity affect the probability of spread of antibiotic resistance in a population of hosts? To address this question, we proposed a multi-scale model, combining within-host dynamics with a stochastic branching process at the between-host scale. We studied how immunity-driven bacterial clustering impacts resistance spread [2].

III. Results

In a microbial population of fixed size, we showed that fast alternations of phases with and without antimicrobial strongly accelerate the evolution of resistance, especially for large populations [1]. Next, we considered microbial populations of variable size, in which we studied the impact of biocidal drugs, that kill microorganisms, and of biostatic drugs, that prevent microorganisms from growing. In both cases, we showed that fast and slow alternations of phases with and without antimicrobial do not permit eradication of the microbial population before resistant mutants fix, while intermediate alternation speeds are effective. Moreover, we showed that biocidal antimicrobials promote resistance more than biostatic ones. Finally, we found a population size-dependent critical drug concentration below which antimicrobials cannot eradicate microbial populations.

In a population of hosts, we demonstrated that immunity-driven bacterial clustering can hinder the spread of resistance [2]. We further showed that the reduction of spread by clustering can be countered when immune hosts are silent carriers, and are less likely to get treated, and/or have more contacts. Our results highlight the importance of interactions between immunity and the spread of antibiotic resistance, and argue in the favor of vaccine-based strategies to combat antibiotic resistance.

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3D imaging of the mammalian microbiota reveals the spatial order of bacteria in the mucosa of intestinal crypts.

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***Short Abstract* — Gaining access to the spatial structure of the microbiota may provide insights into its function. We developed a tissue clearing method for intestinal tissue that is compatible with multiplexed fluorescence *in situ* hybridization imaging of bacteria and mapped the position of 5 relevant bacterial taxa in the crypts of the large intestine. We found a stratified spatial order where bacteria of the Firmicutes and Bacteroidetes phyla segregate from each other. The abundance of Firmicutes bacterial taxa is highly correlated across crypts, suggesting these highly diverse groups cooperate or share a niche. Along crypts, the abundance of all taxa decays with the distance from the lumen at similar rates, suggesting that a general mechanism controls the number of bacteria inside these crevices.**

***Keywords* — microbiome, quantitative imaging, CLARITY, biogeography**

I. Motivation

THE healthy mammalian intestines have an inherent spatial order in which the microbiota is segregated to the lumen. To prevent bacteria from reaching the epithelial layer and crossing it, the host raises chemical and physical barriers that bacteria have evolved to colonize [1]. The synergy between the micro-geography of bacterial consortia and the interactions of bacteria with their environment or other microbes has been studied in synthetic bacterial communities and computational simulations [2], [3]. However, tools for the quantification of the microscopic spatial structure of the intestinal microbiota are lagging.

II. Methods

In this study, we developed an imaging method to quantify the 3D spatial structure of the microbiota of the intestinal mucosa with multiplexed taxonomic resolution. By preserving large tissue samples in whole-mount, our method takes advantage of unrestricted access to image the intestinal mucosa. However, setting up tissue in whole-mount requires that the intestinal tube is open longitudinally, and thus presents two challenges to quantitative imaging: (1) preserving the integrity of the exposed mucus and biofilms, and (2) the natural opacity of thick tissues. To overcome these challenges, we adapted tissue-clearing techniques to intestinal tissue in whole-mount [4], [5]. Additionally, to visualize a diverse microbiota in thick samples, we optimized *in situ* hybridization of 16S ribosomal RNA with signal amplification, and enabled multiplexed imaging of bacteria through spectral imaging.

III. Results

We used our method to image bacteria, mucus and the host epithelium in the ileum and the large intestine. 3D imaging of the mucosa of the large intestine revealed multispecies colonization, and effectively distinguished the location of different taxa in dense cryptal microcolonies. We analyzed computationally the taxonomic composition of dozens of crypts of the large intestine. We performed a hierarchical clustering analysis for the z-scored abundances of each imaged taxon, treating individual crypts as volumetric units. The clustering analysis showed that the distribution of bacterial taxa of the Firmicutes phylum across crypts are the most similar, suggesting these highly diverse groups cooperate or share a niche. Inside crypts, we found a stratified spatial order where bacteria of the Firmicutes and Bacteroidetes phyla segregate from each other. Along crypts, the abundance of bacterial taxa decays at the same rate from the lumen, suggesting that a general mechanism controls the number of bacteria inside these crevices.

IV. Summary

The 3D imaging method we developed to quantify the spatial structure of mucosal microbiota reveals, with unprecedented resolution, key patterns of bacterial association and segregation on the intestinal mucosa, which previously eluded standard approaches like sequencing or thin-section imaging.

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Inferring mechanisms of organelle biogenesis from organelle number and size fluctuations

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Short Abstract — One of the hallmarks of the eukaryotic cell is its organization into distinct spatial compartments known as organelles, which mediate processes critical to cellular function. While the regulation of the abundance and size of organelles plays a profound role in the function and health of a cell, very little is known about the biophysical underpinnings of organelle size regulation. To achieve mechanistic insight into organelle size regulation, we examined three general limits of a comprehensive mathematical model of organelle growth, in which growth either occurs at a constant rate, is regulated by a negative feedback process, or is constrained by a limiting-pool of building blocks. Experimentally, we tested our model on the Golgi apparatus, mitochondria, peroxisomes and lipid droplets in *Saccharomyces cerevisiae*, using quantitative fluorescent microscopy to obtain joint probability distributions of organelle number and size at a single cell resolution. Statistical analyses of our models over a large parameter space and comparison to mutant strains of yeast are consistent with a limiting-pool of organelle building blocks biophysically constraining organelle sizes.

***Keywords* — organelles, quantitative cell biology, stochastic prorcesses.**

I. INTRODUCTION TO ORGANELLE SIZE CONTROL MODELS

ORGANELLES house optimized environments crucial for a diverse array of biochemical processes fundamental to life, from gene transcription in the nucleus to energy production in the mitochondria. Among the key biophysical properties organelles are characterized by are their sizes, which can play a central role in governing organelle output.

Size control of subcellular structures has been best studied quantitatively for the case of linear subcellular compartments, notably cilia and cytoskeletal constituents such as actin [1,2]. These pioneering studies have inspired organelle size control models that generically fall into three categories: organelle sizes can be thought to be set either by a balance of constant growth and decay, by a feedback control process, or by a limiting pool of building blocks that fundamentally constrains growth [3]. Unlike the case of the cilia, a quantitative characterization of the majority of eurkaryotic organelles in terms of organelle size models is lacking.

In order to measure which model best describes the size control for an illustrative set of organelles in *Saccharomyces cerevisiae*, including the Golgi apparatus, lipid droplets, mitochondria, and peroxisomes, we sought to leverage the considerable cell-to-cell statistical fluctuations in organelle numbers and sizes to quantitatively distinguish between different size control models, motivated by our previous work showing that cell-to-cell fluctuations in organelle copy numbers could shed mechanistic insight into organelle biogenesis [4].

II. ORGANELLE SIZE CONTROL MODEL SELECTION

In order to be able to use our experimental data on organelle size and number statistics to distinguish between proposed models of organelle size control, we proceeded by first constructing a general model of stochastic organelle biogenesis and then simulating this model in parameter regimes corresponding to each of the three different size control regimes corresponding to constant growth, feedback control, and limiting pool.

A. Simulating stochastic organelle biogenesis

In our model of organelle biogenesis, both organelle numbers and organelle sizes were considered random variables whose dynamics were captured within a single master equation that could be simulated using the Gillespie algorithm.

In our simulations, we found that each organelle size control model left distinct statistical signatures on the joint probability distributions relating the average organelle size and number of organelles in each simulated cell such that a combination of imaging and genetics would allow for deciphering which size control model best described each organelle under study.

B. Trade-offs in number versus size fluctuations point to the limiting pool model

Experimentally, we found that the cases of the Golgi apparatus, lipid droplets, and mitochondria, the joint distribution of organelle sizes and numbers ruled out all models except the limiting pool model. In the case of the peroxisome, using our mathematical model to interpret joint distributions obtained from strains lacking factors mediating peroxisome fission also ruled out all models except the limting pool model.

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TNFα regulates the tradeoff between speed and accuracy of apoptosis to restrict viral infection

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***Abstract*— Tradeoffs between speed and accuracy are a fundamental concept in cognitive decision theory. It is argued that cellular signaling networks are a biochemical implementation of a cognitive-like process. However, whether cellular decisions are subject to a similar speed-accuracy tradeoff is not known. To address this question, we analyzed how the inflammatory cytokine TNFα alters the cellular decision to apoptose during viral infection. We show that TNFα increases the speed of commitment to apoptosis, preventing viral spread. However, increased speed comes at a cost of spurious death in uninfected cells. Using a Fokker-Planck drift-diffusion framework to analyze Caspase dynamics, we discovered that TNFα changes the potential landscape of Caspase activity, lowering the first passage time to death. Overall, our results show that inflammatory signaling regulates the balance between decision speed and accuracy by tolerating greater error to spare the larger population from infection.**

***Index Terms*— Cell decision making, Apoptosis, Viral infection, Signaling, Quantitative Biology, Cell Fate Committment**

I. Introduction

Decisions are subject to a fundamental tradeoff between speed and accuracy [1]. This notion is so obvious that each of us can relate to it when reflecting on the mundane daily choices we make. Surprisingly, the speed-accuracy tradeoff (SAT) appears ubiquitous in nature. For instance, humans, monkeys, birds, rodents, bees, and ants all exhibit a SAT in individual and collective decision making [1]. The first major clues in deciphering the quantitative basis for a SAT originated when statistical principles from economics were applied to human decision making. Specifically, cognitive decision making was recognized as a sequential sampling problem where the individual evaluates noisy evidence until it accumulates to a decision threshold.

It is argued that cellular signaling pathways are biochemical implementations of a cognitive process [2]. Cells integrate noisy signals from their environment before committing to fate changes such as apoptosis or differentiation. However, it is not known whether cell decisions are subject to a SAT. Are cell fate decisions fundamentally identical to cognitive processes, or is this simply a helpful analogy?

To answer this question we studied how TNFα regulates the cellular decision to apoptose during infection with Herpes Simplex Virus-1 (HSV-1). The rate at which cells apoptose after viral detection is paramount to prevent the virus from completing its lifecycle. As a key immune "warning signal," we hypothesized that TNFα would regulate the cell decision to commit to apoptosis during viral infection.

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II. Results

A. TNFα regulates apoptosis during viral infection

We treated cells with TNFα, infected them with fluorescent-tagged HSV-1 and imaged infection and apoptosis in single cells over 3 days. Cells treated with TNFα dose-dependently increase their speed of commitment to apoptosis during viral infection. This truncated the viral lifecycle and protected neighboring cells from infection, ultimately sparing the population. However, the increase in speed comes at a cost of increased death rate in uninfected cells, or increased false positives. Consistent with other studies of SAT in animals, the speed of commitment to apoptosis is linearly anti-correlated with accuracy. A SAT and its effect on viral spread was further confirmed using different combinations of drugs to artificially lengthen or shorten the time to apoptosis.

B. TNFα alters the potential landscape of Caspase activity

Caspase 8 ascent to a threshold is the major checkpoint before apoptotic inevitability. Therefore, Caspase 8 activity reflects the noisy evidence weighed by the cell when deciding whether to undergo apoptosis. This is analogous to a first passage phenomenon in a drift-diffusion process. We imaged the dynamics of Caspase 8 using an optimized FRET based biosensor, with and without TNFα in thousands of individual cells. We analyzed these Caspase trajectories using a Fokker-Planck drift-diffusion framework and concluded that TNFα changes the potential landscape, thereby reducing the first passage time to death.

III. Conclusion

Overall, our results show how an inflammatory cytokine regulates the balance between cellular decision speed and accuracy. We conclude that cellular signaling networks operate similarly to cognitive processes and are therefore subject to the same fundamental tradeoffs that govern cognitive decision making.

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Molecular Transport by a Propagating Diffusion Barrier

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The *E. coli* MinCDE system has become a paradigm for biological pattern formation. Using an *in vitro* reconstitution assay, we show that MinDE self-organization can spatially regulate a variety of functionally completely unrelated membrane proteins into patterns and gradients. MinDE concentration waves induce a direct net transport of tightly membrane-attached molecules. Using highly controllable, membrane-bound DNA nanostructures we investigate the active transport through a traveling diffusion barrier in detail. This phenomenon may be a general mechanism of spatiotemporal regulation in cells and can be employed to transport artificial cargo in synthetic biology applications.

I. The MinDE System is a Generic Spatial Cue for Membrane Protein Distribution *in vitro*

Spatiotemporal organization is key to transform a "pool of molecules" into a functional cell capable of regulating complex tasks, such as cell division and chromosome segregation. In bacteria many of these functions are executed by reaction diffusion systems, of which the *Escherichia coli* MinCDE system is the most prominent and has become a paradigm for pattern formation in biology. Based on the ATPase MinD, its activator MinE and the membrane as a reaction matrix this minimal biological oscillator defines midcell in *E. coli*. The third protein, MinC, an inhibitor of FtsZ, merely follows the MinDE oscillations resulting in a temporal concentration gradient that restricts the main divisome protein FtsZ to midcell.

Here, we found that the ATP-consuming MinDE oscillations may play a role far beyond constraining MinC/FtsZ localization. Using a well-established *in vitro* reconstitution assay on supported lipid bilayers [1] we show that MinDE oscillations are able to spatiotemporally regulate a variety of functionally unrelated membrane proteins [2]. Intriguingly, the ATP-driven MinDE self-organization constitutes a dynamic diffusion barrier that induces directed and active net transport of lipid-anchored proteins, establishing large-scale gradients on the membrane. When co-reconstituted in rod-shaped microcompartments MinDE pole-to-pole oscillations drive counter-oscillations of unrelated membrane-bound proteins resulting in time-averaged protein gradients that are minimal at compartment poles, indicating protein enrichment in the compartment middle [2]. These findings imply that MinDE is able to position a much larger set of proteins than previously known, thereby contributing, independent of MinC, to division site selection by prepositioning divisome proteins to midcell.

II. Understanding the Molecular Transport Using Membrane-Anchored DNA Nanostructures

We employ this simplistic transport mechanism for positioning a synthetic cargo: membrane-anchored DNA nanostructures. By varying the number of membrane anchors on the highly controllable DNA origami [3] we determined the influence of the cargo mobility on its spatiotemporal positioning by MinDE. We find that the diffusion coefficient and the drag of the target molecule determine the strength of the spatiotemporal regulation. Using this knowledge, we are able to sort and spatially separate DNA origami according to the number of membrane anchors by MinDE self-organization. We also use patterned supported lipid bilayers to guide the MinDE-dependent transport achieving defined and controlled transport of the DNA origami. We will further employ single-particle tracking of DNA origami to better understand the mechanism of a propagating diffusion barrier. In the future the DNA origami will serve as a modular platform to anchor additional molecules generating new active systems in synthetic biology applications.

III. Conclusion

In summary, the MinDE-dependent regulation of membrane-bound molecules by a propagating diffusion barrier can be seen as an archetypal physicochemical mechanism based on two proteins only. We speculate that also other bacterial or eukaryotic reaction diffusion systems are capable of regulating a large set of proteins by similar non-specific interactions, hinting towards a generic mechanism to couple ATP consumption to protein gradient formation. Furthermore, this simplistic transport mechanism serves to position membrane-bound molecules in synthetic biology applications.

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Probing Macromolecular Crowding in the Cell Nucleus

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***Short Abstract* — The cell is filled with macromolecular complexes that must navigate through a crowded environment to perform their functions. Here, we are developing the use of genetically-encoded multimeric nanoparticles (GEMs) (described in [1]) in live fission yeast cells to study the rheology of the nucleus. GEMs are spherical protein structures, ranging from 20 to 50 nm in diameter, that are labeled with fluorescent proteins. Analysis of the diffusive-like motion of these particles in the cell provides a measurement of local crowding within subcellular compartments. We find that the addition of sorbitol, an osmotic agent, decreases the volume of the cytoplasm and the nucleus at a comparable fraction and similarly decreases GEMs diffusion in both compartments. We also find that GEMs diffusion does not change significantly during the cell cycle. Finally, we demonstrate that inhibiting ribosome biogenesis increases GEMs diffusion in both the cytoplasm and the nucleus.**

***Keywords* — Macromolecular crowding, nucleus, microrheology, GEMs**

I. Background

MACROMOLECULAR crowding is a fundamental physical property within all living cells, and yet, study of this basic cellular feature remains dismally sparse. Macromolecular crowding is the phenomenon in which the properties of molecules are altered in the presence of high concentrations of macromolecules (e.g. proteins, nucleic acids, carbohydrates) [2]. In particular, the cell nucleus is an important organelle with which to study macromolecular crowding and its relationship to nuclear function.

In eukaryotes, the nucleus is exclusive site of multiple essential cellular processes. For example, ribosome biogenesis occurs within the nucleolus, the largest structure in the nucleus. In addition, the nucleus contains chromosomes which encode the entire genetic information of the cell. Thus, the nucleus is also the sole location of transcription.

The fission yeast *Schizosaccharomyces pombe* is an ideal system to study macromolecular crowding in the nucleus and its impact on nuclear function. *S. pombe* is a unicellular organism that has a nucleus with organizational hallmarks found in higher eukaryotes. Importantly, we have successfully implemented GEMs, a novel tool to probe macromolecular crowding developed by Delarue et al. [1], for use with *S. pombe*. GEMs, or genetically-encoded multimeric nanoparticles, belong to a class of methods to study the rheological properties of a medium termed passive microrheology. Using single-particle tracking of GEMs, diffusion coefficients of these nanoparticles can be derived in order to infer crowding *in vivo*.

The overall goal of this project is to identify the macromolecules that contribute to crowding in the nucleus and to define the relationship between crowding and nuclear function. This research will promote better understanding of a fundamental physical property in biology and its role in essential cellular processes.

II. Results

A. Reducing nuclear volume by sorbitol treatment decreases GEMs diffusion in the nucleus

Sorbitol, an osmotic agent, decreases nuclear volume at a comparable proportion to the whole cell volume. Further, sorbitol treatment reduces GEMs diffusion of 20-50nm particles in the cell nucleus.

B. GEMs diffusion is comparable throughout the cell cycle

During the cell cycle, the cell undergoes several physical changes including chromosome compaction during metaphase. Using microtubule architecture to determine cell cycle stage, 40nm-GEMs diffusion is comparable in both the cytoplasm and nucleus between interphase and metaphase.

C. Inhibiting ribosome biogenesis increases GEMs diffusion in both the cytoplasm and the nucleus

Ribosomes are the most abundant macromolecule in the cell, and recent studies show they are the major crowding agent in the cytoplasm [1]. Using Rbin-1 [3], a potent and reversible inhibitor of ribosome assembly, GEMs diffusion increases in both the cytoplasm and the nucleus.

III. Conclusion

These results indicate that crowding may not be changed significantly throughout the cell cycle and that ribosomes may also be a major crowding agent in the nucleus. In future studies, we hope to characterize the crowding factors in the nucleus and determine biological situations in which crowding may change.

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Steering a bacterial pathogen through the phenotype space of multidrug resistance

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***Abstract*— Evolved resistance to one antibiotic may be associated with "collateral" sensitivity to other drugs. Using large-scale experimental evolution, we measure phenotypic profiles of collateral sensitivity and resistance for more than 1000 mutant-drug combinations in *E. faecalis*, a gram-positive opportunistic pathogen. We find that collateral effects are pervasive but highly heterogeneous, even for populations selected with the same drug. To exploit these profiles, we use a Markov Decision Process to assign an optimal drug to every possible resistance profile. Using both simulations and experiment, we show that these optimal drug policies lead to aperiodic drug sequences capable of maintaining long-term drug sensitivity at the expense of short-term periods of high resistance.**

I. Introduction

Collateral sensitivity occurs when a microbial population evolves resistance to a target drug while simultaneously exhibiting increased sensitivity to a different drug. Recent studies in bacteria indicate that collateral effects may slow resistance evolution (see, for example, [1], [2]), though collateral profiles have also been shown to be highly heterogeneous [3], [4]. The picture that emerges is enticing, but complex; while collateral effects offer a promising new dimension for improving therapies, the design of drug cycling protocols is an extremely difficult problem that requires optimization at multiple scales. In this work, we take a step towards answering these questions by investigating how drug sequences might be used to slow resistance in a simplified, single-species bacterial population. We show that even in this idealized scenario, intuitive cycling protocols–for example, sequential application of two drugs exhibiting reciprocal collateral sensitivity–are expected to fail over long time periods, though mathematically optimized policies can maintain long-term drug sensitivity at the price of transient periods of high resistance. As a model system, we focus on *E. faecalis*, a gram-positive opportunistic bacterial pathogen. *E. faecalis* are found in the gastrointestinal tracts of humans and are implicated in numerous clinical infections, ranging from urinary tract infections to infective endocarditis [5].

II. Results

By combining parallel experimental evolution of *E. faecalis* with high-throughput dose-response measurements, we characterize collateral sensitivity and resistance profiles for more than 60 populations evolved to 15 different antibiotics and 7 additional stressors, including biocides and preservatives. We find that collateral effects are surprisingly pervasive, even between drugs or stressors from unrelated classes. At the same time, the sensitivity profiles–while highly heterogeneous–cluster into groups characterized by selecting drugs from similar drug classes.

To exploit the statistical structure in the resistance profiles, we develop a simple mathematical framework based on a Markov Decision Process (MDP) to identify optimal antibiotic policies that minimize resistance. These policies yield drug sequences that can be tuned to optimize either short-term or long-term evolutionary outcomes, and they codify the trade-offs between instantaneous drug efficacy and delayed evolutionary consequences. Stochastic simulations reveal that these policies outperform previously proposed random and simple periodic dosing schemes, and experiments confirm that judicious sequences of drugs can be used to simultaneously sensitize the population to multiple antibiotics.

III. Conclusion

Our results point to widespread collateral effects of drug resistance in an opportunistic pathogen. Despite the apparent unpredictability of collateral effects at the level of individual mutants, we show how these profiles can be incorporated into a simple mathematical framework that optimizes drug protocols while accounting for effects of both stochasticity and different time horizons. Perhaps most strikingly, our results indicate that it may be possible to constrain resistance over surprisingly long timescales using optimized, aperiodic drug cycles that include (locally) sub-optimal steps where the drug is instantaneously less effective but shepherds the population to a more vulnerable evolutionary state.

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Modeling the Regulation of Cellular Quiescence Depth by Circadian Clock Proteins

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***Short Abstract* — Reactivating quiescent cells to divide is fundamental to tissue repair and homeostasis. Like sleep that has shallow and deep stages, cellular quiescence exhibits graded depths featuring varying rates and speeds of reactivation upon growth signals. Here we studied the modulation of quiescence depth by circadian clock. By modeling a library of potential links from circadian proteins to the Rb-E2F-cyclin/Cdk gene network, we identified and experimentally confirmed a novel converging effect of circadian proteins Cry and Rev-erb to downregulate G1 cyclin/Cdk activities, which in turn increases the activation threshold of the Rb-E2F bistable switch and deepens cellular quiescence accordingly.**

***Keywords* — Quiescence depth, heterogeneity, circadian clock, Rb-E2F pathway, bistable switch, activation threshold.**

I. INTRODUCTION

QUIESCENCE is a non-proliferative cellular state in the body. Reactivation of quiescent cells (e.g., fibroblasts, lymphocytes, and stem cells) to enter the cell cycle is fundamental to tissue regeneration and homeostasis. Often referred to as the "G0" state, quiescence is in fact not a single uniform state but with graded depths [1]. Shallow quiescent cells (e.g., muscle and neural stem cells post tissue injury) exhibit a higher tendency to revert to proliferation upon stimulation than deep quiescent cells. Dysregulation of cellular quiescence depth can lead to a range of hyper- and hypo-proliferative diseases including cancer and aging.

Circadian clock modulates biological rhythms in the body responding to daily environmental changes. Cryptochrome (Cry) and Rev-erb are two important circadian proteins that separately inhibit their common transcriptional activator Bmal1/Clock, forming two negative feedback loops that are responsible for generating cellular circadian rhythm [2]. Circadian clock affects a wide range of cellular activities such as proliferation and metabolism, and is increasingly recognized as a critical modulator of many physiological and disease processes. However, little is known about whether and how circadian clock influences cellular quiescence.

II. RESULTS

Here we focused on testing the roles of circadian proteins Cry and Rev-erb in modulating cellular quiescence depth. Experimentally, we found that activating Cry and Rev-erb in quiescent rat embryonic fibroblasts upregulated oncogene c-Myc and downregulated cyclin-dependent kinase (Cdk) inhibitor $p21^{Cip1}$, respectively, as expected from literature [3, 4]. However, in both cases, cells did not move to a 'shallower' quiescent state as expected but a 'deeper' state, requiring stronger serum stimulation and a longer time to exit quiescence.

To identify the missing links from circadian proteins Cry and Rev-erb to the regulation of quiescence depth, we systematically modeled a library of regulatory network topologies connecting the two circadian proteins to the Rb-E2F-cyclin/Cdk gene circuit that controls quiescence exit to proliferation [5]. Our modeling search predicted a converging pattern: Cry and Rev-erb both downregulate the kinase activity of cyclinD/Cdk4,6 complex (but not cyclinE/Cdk2), which overrides concurrent changes in Myc and $p21^{Cip1}$ activities, resulting in a net effect of significantly increasing the activation threshold of the Rb-E2F bistable switch and deepening quiescence accordingly [6].

Our follow-up experiments confirmed our model predictions and demonstrated that continuously increasing Cry and Rev-erb activities gradually increased the Rb-E2F activation threshold and quiescence depth by downregulating cyclinD/Cdk4,6, acting like a dimmer switch.

III. CONCLUSION

Our findings suggest a novel mechanistic role of circadian clock proteins in modulating the depth of cellular quiescence, which may have implications in varying potentials of tissue repair and regeneration in different times of the day.

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Ultra-sensitive and fractal-like growth of cells

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Short Abstract — **Every organism has a range of temperatures at which it can grow and reproduce. What sets the boundary between habitable and inhabitable temperatures is poorly understood. We used the budding yeast, *Saccharomyces cerevisiae*, to address this question. We discovered that yeasts can, in fact, grow at high temperatures at which they are conventionally believed to not grow. We discovered that cells at high temperatures can remain still for days, before stochastically transitioning into a growing phase and dividing. This leads to fractal-like structures and cell-growth that is highly sensitive to minute changes of metabolites. A mathematical model explains these phenomena.**

Keywords — **Unconventional cell-growth, life-and-death transition, temperature, stochastic growth.**

I. Background

THE conventional view of cell growth is that when the temperature is above a certain value, cells take longer times to divide than they would at lower temperatures and that when the temperature is sufficiently high, cells fail to divide and may eventually die [1]. This view applies to a wide range of organisms, including the budding yeast, *Saccharomyces cerevisiae*. In this textbook picture [1], as the temperature increases, the growth rate decreases and eventually becomes zero at sufficiently high temperatures. Despite being widely accepted and - since we can also experimentally reproduce this picture - evidently true, we realized that this picture is, in fact, an illusion. By examining how yeast cells grow at high temperatures, we discovered a revised picture of cellular growth at high temperatures, including at temperatures for which the conventional view states that yeasts cannot grow. In the process, we discovered how the misleading conventional view arises.

II. Summary of Results

By growing haploid, laboratory-standard budding yeasts at high temperatures (e.g., 42C), we observed that populations of yeasts do not seem to grow or divide, in accordance with the textbook picture. Yet, by studying individual cells, we discovered that cells stochastically transition from being inactive (not growing or dividing) to an active (growing and dividing) state. Crucially, in some cases, cells might wait days to weeks before transitioning to a growing phase. Once the first cell transients into an active state and divides, its subsequent generations have higher chances of diving. Surprisingly, the rate of cell-growth at high temperatures, after the transition, can be nearly the same as the growth rate at much lower, optimal temperature (~30C). Due to the stochastic nature of this phenomenon, we find that cells' growth rate is highly variable, even for the same temperature. As a result, we discovered that for a range of temperatures, the growth rate of cells is only weakly correlated with temperature (i.e., nearly independent of temperature). This is in contrast to the conventional picture in which the growth rate decreases as temperature increases above an optimal value. By tuning external metabolites and cost of expressing spurious genes, we could tune the growth behaviors. For example, we found that a minute change in metabolite concentrations outside the cells can make all the difference between having cells grow or not grow at all at the same high temperature - a feature that we call ultra-sensitive growth. Using microscopy, we also discovered that the cells can form fractal-like structures at high temperatures.

We developed a mathematical model to describe both the ultra-sensitive and fractal growth. A consequence of this model, which we experimentally verified, is that yeasts can, in fact, remain viable at high temperatures for an arbitrarily long time despite essential proteins - which are thought to be necessary for cell-division and viability - unfolding at these temperatures.

III. Conclusions

We discovered that cells can actually grow at much higher temperatures than expected from the textbook picture and that the apparent decrease in the growth rate as temperature increases is an illusion that stems from not studying individual cells and not waiting long enough to observe individual cells that suddenly start to grow after a long period (days to weeks) of stasis.

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