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How to train your microbe: methods for dynamically characterizing gene networks

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Abstract

Gene networks regulate biological processes dynamically. However, researchers have largely relied upon static perturbations, such as growth media variations and gene knockouts, to elucidate gene network structure and function. Thus, much of the regulation on the path from DNA to phenotype remains poorly understood. Recent studies have utilized improved genetic tools, hardware, and computational control strategies to generate precise temporal perturbations outside and inside of live cells. These experiments have, in turn, provided new insights into the organizing principles of biology. Here, we introduce the major classes of dynamical perturbations that can be used to study gene networks, and discuss technologies available for creating them in a wide range of microbial pathways.

Introduction

Static perturbations have yielded fundamental biological insights. However, gene networks are inherently dynamic: they sense and respond to extra- and intracellular stimuli [1–4], change internal state [5–9], and regulate cell-level functions [10–13] in time. Accordingly, precise temporal perturbations (and observations) are needed to fully understand gene network structure and function [14,15].

An increasingly common approach is to use a continuous culture device to characterize the response of a network to time-varying extracellular perturbations [16–34]. For example, when galactose is introduced in the absence of the preferred carbon source glucose, *Saccharomyces cerevisiae* rapidly induce transcription of galactose utilization (*GAL*) genes

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(Fig. 1a) [35]. If glucose is subsequently added, glucose utilization genes are quickly activated while *GAL* genes are repressed [22]. To investigate the dynamics of this switch, Hasty and coworkers combined a custom microfluidic device (Fig. 1b) with time-lapse fluorescence microscopy to monitor the expression dynamics of fluorescent protein (FP)-tagged Gal1 in yeast grown in constant galactose and fluctuating glucose (Fig. 1c) [21]. A mathematical model revealed that transcriptional regulation alone could not account for the speed of the response (observed as the amplitude of the Gal1 wave) (Fig. 1c), leading the group to identify a new interaction wherein glucose induces degradation of *GAL* mRNAs, including *GAL1* (Fig. 1a, c) [21]. Follow-up studies have demonstrated that this rapid post-transcriptional regulatory interaction provides a fitness advantage to yeast grown in fluctuating glucose environments [17,36].

Dynamic intracellular perturbations can be made by expressing network genes from non-native promoters [37–47]. In response to starvation, a fraction of *Bacillus subtilis* differentiate into metabolically inert, stress-resistant spores. Sporulation is induced by a multi-step network resulting in phosphorylation and activation of the master transcription factor (TF) Spo0A. A supra-threshold concentration of phosphorylated Spo0A (Spo0A~P) was long thought to be sufficient to induce sporulation [48,49]. However, Fujita and coworkers recently demonstrated that gradual Spo0A~P accumulation is also essential by expressing *spo0A* and its direct kinase *kinC* from different chemically-inducible promoters (Fig. 1d), such that the rate of accumulation and final level could be independently controlled (Fig. 1e, f) [39]. Furthermore, after the onset of starvation, Spo0A~P levels have been found to ramp up via a series of cell cycle-linked pulses of increasing amplitude [50,51], though the role of these pulses are poorly understood. Therefore, an ideal experiment would be to express *spo0A* and *kinC* from inducible promoters and apply time-varying inducer levels to program Spo0A~P to increase in pulsatile and non-pulsatile manners, while monitoring Spo0A~P regulated genes and sporulation phenotypes.

Historically, there have been no technologies for programming precisely defined gene expression or protein activity dynamics ‘on demand’. However, recent studies have overcome this limitation by combining mathematical modeling and computational control with custom hardware and time-varying chemical inducers [24,52–54], or genetically-encoded light switchable proteins (optogenetic tools) [55–57]. In several cases, the resulting perturbations have been used to reveal new dynamical properties of promoters and gene circuits [24,54,55]. Here, we introduce the different dynamical perturbations that can be used to study gene networks, and review recent technologies available for generating them.

Biological signals - an engineering perspective

Borrowing terminology and concepts from electrical engineering, we refer to genetically-encoded elements with basic functions (e.g. promoters, kinases, or gene circuits) as biological “components” [15]. The primary function of components is to transform input signals into outputs that can be relayed to other components (Fig. 2). For example, a regulated promoter may convert an active TF concentration into a transcription rate. An “effector” is an experimentally tractable agent, such as light (Fig. 2a) or osmotic pressure (Fig. 2b), that perturbs a biological signal(s) in a well-understood manner. “Transducers”,

such as membrane receptors or soluble TFs, convert effector signals into biological signals. Organism-level functions, such as metabolism or sporulation, are controlled by biological “systems”, or networks of interconnected components. Alternatively, “system” can be used to describe any biological element(s) under study.

Ideally, if one understood how all components in a given system were connected, and how each transformed inputs into outputs, one would fully understand the system. However, the performance of some components can change depending on the component(s) they are connected to [58,59]. Additionally, components can interact indirectly by competing for shared resources or altering a property of the host cell that affects component performance, such as growth rate [15,60–65]. For example, highly expressed mRNAs can sequester ribosomes, reducing expression level and increasing expression stochasticity of other genes [64], while additional operator sites can compete for TF binding and ‘buffer’ regulatory signals at other promoters [58,65]. Synthetic biologists are currently developing frameworks for classifying and characterizing such context-dependent and indirect interactions so that systems-level processes can be understood as a straight-forward function of the components, and several key properties of the host cell and environment [15,66–68].

Dynamical perturbations - signals

To dynamically characterize a system, one will typically expose it to one or more input signals (Table 1) and measure how it transforms them into outputs. In biological research, the most common inputs are ‘constant’ and ‘step’ chemical effector signals (Table 1). Characterization of the output in response to step changes in effector can be sufficient to capture the full dynamics of some systems. For example, Olson and coworkers measured expression of the reporter gene GFP over time to characterize the transcriptional output of the CcaS-CcaR bacterial two component system (TCS) (Fig. 2a) to step increases and decreases in the effector green light [55]. These experiments revealed that CcaS-CcaR has a 5-minute delay, followed by a 1–28 minute transition from the initial to 50% of the final transcription rate, depending on the light intensity and sign of the step change. The experimental data were used to construct and parameterize a simplified ordinary differential equation model that quantitatively predicts output in response to much more complex dynamical inputs over 12 hours with high accuracy [55].

Step response characterizations can be insufficient for systems containing feedback or feed-forward loops, which can have more complex dynamics such as outputs that depend on the rate of change of the input. Such systems are better characterized using ‘ramps’, or gradual transitions between two input levels (Table 1) alongside steps or other signals [16,19,27,30,69]. For example, a recent study revealed that step increases of NaCl and ethanol activate the *B. subtilis* general stress response, but that slow linear ramps induce more stress-specific responses [16] (Fig. 3c). This rate-discrimination occurs via a slow negative feedback loop. The general stress response is activated by the alternative sigma factor σ^B , which is sequestered and inactivated by the anti- σ^B factor RsbW. If stress increases quickly, the anti- σ^B factor RsbV is rapidly dephosphorylated, allowing it to bind and de-activate RsbW, liberating σ^B to activate target promoters. However, σ^B also induces transcription of *rsbW*, a relatively slow process that inhibits σ^B . If stress increases

slowly, RsbW is produced in substantial quantities before σ^B activity rises appreciably, thus repressing the general stress response and permitting activation of σ^B -independent response pathways (Fig. 3c).

Periodic inputs, such as sine or square waves, can be used to decrease measurement noise, place bounds on the kinetic properties of rate-limiting components, and identify processes that dominate the dynamical behavior of a system [21,24,25,28,29,54,55,70–73] (Table 1). For example, Van Oudenaarden and coworkers used square waves of NaCl of increasing frequencies to investigate the *Saccharomyces cerevisiae* osmotic stress response (Fig. 2b) [28]. Hog1 is a mitogen activated protein kinase (MAPK) that stimulates glycerol production, which increases membrane turgor, in response to increased osmotic pressure. The dynamical measurements revealed that the Hog1 pathway contains a previously unknown fast (5-minute) post-transcriptional response as well as a slower (15-minute) transcriptional response, ensuring that osmotic challenges are met quickly and that the response is sustained. A similar approach revealed that a Sln1-based signaling branch transmits effector signals to Hog1 at least twice as fast as the seemingly redundant Sho1 branch [29] which may produce a more reliable and faster response to mild osmotic stresses [74].

Effectors and transducers

Effectors are the signal carriers used to make dynamical perturbations. The concentration of an ideal effector would be easy to control externally and adjust faster than the molecular biological processes being studied [75]. Ideally, a change in effector concentration would also be transformed into a biological signal instantaneously and the effector will not cross-talk with off-target transducers or pathways [15].

The simplest approach is to use a ‘native effector’ - one to which a system being studied in its evolved context naturally responds. In recent examples, NaCl and ethanol signals were used to study the σ^B system [16], alpha-methyl-DL-aspartate signals for the *Escherichia coli* chemotaxis system [30], and glucose [17,21,36], KCl and H₂O₂ [24,25], NaCl and sorbitol [27–29,34], and α -factor [31–33] signals for the *S. cerevisiae* glucose-galactose, general stress response, Hog1, and mating systems, respectively. Though convenient, native effectors are often subject to regulated import, catabolism [76–78], cross-talk [79], or other interactions with the cell that can confound analyses.

A carefully chosen non-native effector/transducer pair, such as an engineered promoter that responds to a gratuitous-like inducer [76,80–85], can overcome many limitations of native effectors. The most common approach for investigating microbial gene network dynamics has been to isolate the effects of the expression of a single gene by replacing the native promoter with a chemically-inducible version in bacteria [37,39–45] (Figure 1d–f).

However, all chemical effectors have limitations. In batch culture, cell density increases over time, thus reducing the effector concentration per cell. If effector is not in excess, the strength of the perturbation may therefore change during the experiment. Additionally, though step increases can be generated by spiking in chemicals, step decreases require washing or dilution, which are slow and can introduce unwanted physiological

perturbations. Finally, many chemicals must traverse one or more membranes before becoming accessible or inaccessible to the transducer. Transport introduces effector-specific time delays that can be poorly defined and put speed limits on the biological signals ultimately being used for characterization.

Light is an ideal effector because it can be precisely controlled in the wavelength, intensity, temporal, and spatial dimensions, suffers no transport delays, and has minimal cross-reactivity in most laboratory organisms. Recently, light-activated and de-activated transcriptional regulatory systems with wavelength specificities from the blue (430nm) to far red (756 nm) have been engineered in *E. coli* [86–92]. The blue activated/dark-deactivated pDawn [87], green activated/red de-activated CcaS-CcaR (Fig. 2a) [92], and red de-activated/far red activated Cph8-OmpR [92] systems are TCSs with the desirable features of streamlined plasmid encoding, portability to most strains, low leaky transcription in the deactivating condition, and near or exceeding 100-fold light response. However, pDawn has a 2 hour delay prior to responding to blue light input, limiting its use for perturbing gene expression dynamics, while CcaS-CcaR and Cph8-OmpR are useful due to their 5 and 0 minute delays and programmable output dynamics [55]. Ultraviolet B (280 nm) [93] and blue [94,95] activated, and red activated/far red de-activated [96] transcriptional tools based on light-switchable protein dimerization and the yeast two-hybrid system have been engineered for *S. cerevisiae*, while CcaS-CcaR and the blue light inducible *vvd* promoter have been used to express heterologous genes in *Synechocystis* PCC6803 [97] and *Neurospora crassa* [98], where they are native. Additionally, blue/dark switchable proteolysis [99,100] and nuclear/cytoplasmic translocation [101] systems have been engineered in *S. cerevisiae*. Combined with complementary advances in optical hardware, these optogenetic tools are making light an increasingly practical replacement for chemical effectors.

Hardware for controlling optogenetic tools in batch culture

Batch experiments are simple, inexpensive, and scalable. Several batch instruments have recently been designed for dynamical optogenetic perturbations. One example is the “Light Tube Array” (LTA) (Fig. 3a), a shaker/incubator-mounted device that holds 64 culture tubes, each above independently programmable blue, green, red and far red light emitting diodes (LEDs). Olson and coworkers used the LTA to characterize CcaS–CcaR and Cph8–OmpR [55]. By combining the resulting mathematical models with a computational optimization algorithm to design light signals, the group could directly program sophisticated gene expression output signals from these TCSs. For example, using green light signals to create linear ramps and sinusoids of the tetracycline repressor (TetR) via CcaS-CcaR, the group demonstrated that the widely used $P_{\text{LtetO-1}}$ promoter linearly transforms high TetR inputs into low transcriptional outputs with a 7 minute delay over a 4.5-fold range of TetR concentrations [55] (Fig. 3a). 96-well plate-based optogenetic instruments have also been built [102] and used to program more basic gene expression dynamics in *E. coli* and yeast [56,103]. Plate-based devices should be compatible with the method of Olson and coworkers, while increasing throughput relative to the LTA.

Continuous Culture

Continuous culture instruments overcome many limitations of batch by maintaining constant nutrient and effector levels and growth rates (chemostats) or cell densities (turbidostats), and are amenable to automated dynamical perturbations and both population and single cell measurements. However, the use of continuous culture in microbiology research has been limited by the fact that most devices are proprietary, expensive, and inflexible.

Recently, Klavins and coworkers designed the Flexostat (Fig. 3b), an open source, 8-chamber turbidostat built from 3D-printed parts and standard electronics that can be assembled by non-experts for under \$2,000 [104]. A computer feedback controller maintains desired culture densities by adjusting the media flow rate in response to optical density. GFP expression can be measured continuously using an embedded fluorimeter, and a 15 mL chamber volume permits ~100 μ L samples to be effluxed every ~1 minute for off-line analysis. The Flexostat can also generate constant or time-varying chemical effector signals by drawing from one media source or alternating between two (Fig. 3b). However, because the flow rate matches the population doubling time, one doubling is required to dilute the effector concentration by 50%.

Blue LEDs were recently combined with a custom chemostat and integrated fluorescence microscope to control a blue light activated promoter while measuring gene expression in single *S. cerevisiae* cells [57]. By combining the method of Olson and coworkers [55] (Fig. 3a) with continuous culture devices, one could perturb and observe gene network dynamics with very high temporal resolution in controlled growth environments.

Microfluidics

Due to their nanoliter volumes, which permit media to be changed in seconds, and compatibility with time-lapse fluorescence microscopy, microfluidic devices are increasingly being used to characterize gene network dynamics in single cells [105,106]. While groups often design custom devices for specific experiments, a single commercial platform was used to generate steps and ramps in the sB study (Fig. 3c) [16], anhydrotetracycline steps and pulses to characterize an engineered TCS with negative feedback [107], a-factor [31,32] and phosphate starvation steps and pulses [108], and auxin [109] and KCl [73] square waves in recent studies of natural and synthetic yeast networks. A comparison of devices used in published yeast studies is given in a recent report of a long-term microchemostat for monitoring yeast aging [110].

Similar to the optogenetic programming of GFP and TetR signals in *E. coli* [55] (Fig. 3a) several groups have recently used microfluidics and chemical effectors to program FP expression [52,53] and TF activity signals in *S. cerevisiae* [24,25,54]. In the absence of stress, the TF Msn2 is maintained in the cytoplasm via phosphorylation by cyclic AMP (cAMP)-dependent protein kinase A (PKA) [111]. Different stresses induce Msn2 to dynamically translocate into and out of the nucleus, likely due to cAMP oscillations [112]. Information about the identity (e.g. glucose starvation, osmotic shock, or oxidative stress) and magnitude of the stress are encoded in the duration, amplitude, and frequency of Msn2 nuclear localization events, and different stresses result in different promoter responses

[24,113]. To examine how Msn2 dynamics can be decoded by different promoters, O'Shea and coworkers combined PKA variants that are inhibited by the synthetic ligand 1-NM-PP1 with custom microfluidic devices. By applying different 1-NM-PP1 pulse sequences, the group programmed artificial Msn2 nuclear localization pulses of variable amplitude and duration, and oscillations of variable frequency [24,25,54]. Combined with mathematical modeling, these results showed that promoters with different TF binding affinities and activation kinetics can respond differently to the same TF dynamics, and that different TF dynamics preferentially activate different promoters [54]. The encoding of regulatory information in TF dynamics may sidestep the need evolve additional TFs [54], enable proportional induction of large numbers of genes [114], coordinate the timing of developmental decisions [51,115], and have other benefits [1].

To better understand gene regulatory dynamics, methods for directly programming biological signals could further be combined with single cell, transcriptomic, proteomic, and CRISPR/Cas-based approaches. For example, responses to 1-NM-PP1 driven Msn2 dynamics could be measured at the proteome-level using a recent chemostat array and the GFP-tagged yeast genome library [116]. Alternatively, the localization dynamics of Msn2 or other dynamically regulated TFs [117] could be directly programmed with light [101] at the single cell level using modern projection and computer control technologies [118,119]. Chemical and optogenetic methods for programming gene expression dynamics could also be combined with RNA-guided CRISPR/Cas transcriptional activation/repression technologies [120–123] and FP-reporters or -omics analyses to send dynamical gene expression signals through virtually any gene network while observing how those signals propagate and affect phenotypes.

Conclusion

Genetic, hardware, and computational tools for making precise dynamical perturbations to microbial gene networks are advancing rapidly. When combined with modern observational methods and mathematical modeling, the perturbations enabled by these tools will likely revolutionize our understanding of the path from genotype to phenotype in a wide range of pathways and organisms.

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References

- * Of special interest
- * Of outstanding interest
- **1. Levine JH, Lin Y, Elowitz MB. Functional roles of pulsing in genetic circuits. *Science*. 2013; 342:1193–1200. This excellent review describes the diverse modes of dynamical gene regulation that have recently been discovered across biology, and the unique functional roles that such dynamics can play. [PubMed: 24311681]

2. Alon, U. An Introduction to Systems Biology. Chapman & Hall/CRC; 2007.
3. Yosef N, Regev A. Impulse control: temporal dynamics in gene transcription. *Cell*. 2011; 144:886–896. [PubMed: 21414481]
4. Purvis JE, Lahav G. Encoding and decoding cellular information through signaling dynamics. *Cell*. 2013; 152:945–956. [PubMed: 23452846]
5. Luscombe NM, Babu MM, Yu H, Snyder M, Teichmann SA, Gerstein M. Genomic analysis of regulatory network dynamics reveals large topological changes. *Nature*. 2004; 431:308–312. [PubMed: 15372033]
6. Komurov K, White M. Revealing static and dynamic modular architecture of the eukaryotic protein interaction network. *Mol Syst Biol*. 2007; 3:110. [PubMed: 17453049]
7. Bandyopadhyay S, Mehta M, Kuo D, Sung M-K, Chuang R, Jaehnig EJ, Bodenmiller B, Licon K, Copeland W, Shales M, et al. Rewiring of genetic networks in response to DNA damage. *Science*. 2010; 330:1385–1389. [PubMed: 21127252]
8. Ideker T, Krogan NJ. Differential network biology. *Mol Syst Biol*. 2012; 8:565. [PubMed: 22252388]
9. Park Y, Bader JS. How networks change with time. *Bioinformatics*. 2012; 28:i40–8. [PubMed: 22689777]
10. Barabási A-L, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet*. 2004; 5:101–113. [PubMed: 14735121]
11. Seshasayee ASN, Bertone P, Fraser GM, Luscombe NM. Transcriptional regulatory networks in bacteria: from input signals to output responses. *Curr Opin Microbiol*. 2006; 9:511–519. [PubMed: 16942903]
12. Ni L, Bruce C, Hart C, Leigh-Bell J, Gelperin D, Umansky L, Gerstein MB, Snyder M. Dynamic and complex transcription factor binding during an inducible response in yeast. *Genes & Development*. 2009; 23:1351–1363. [PubMed: 19487574]
13. Laub MT, Shapiro L, McAdams HH. Systems biology of *Caulobacter*. *Annu Rev Genet*. 2007; 41:429–441. [PubMed: 18076330]
14. Alexander RP, Kim PM, Emonet T, Gerstein MB. Understanding modularity in molecular networks requires dynamics. *Sci Signal*. 2009; 2:pe44–pe44. [PubMed: 19638611]
- *15. Olson EJ, Tabor JJ. Optogenetic characterization methods overcome key challenges in synthetic and systems biology. *Nature Chemical Biology*. 2014; 10:502–511. This perspective piece discusses how optogenetics can be used to understand systems-level biological phenomena at the level of the basic components.
- **16. Young JW, Locke JCW, Elowitz MB. Rate of environmental change determines stress response specificity. *Proc Natl Acad Sci US A*. 2013; 110:4140–4145. In this study, a commercial microfluidic system is used to generate ramps and steps of ethanol and NaCl in order to identify rate-sensing in the *B. subtilis* general stress response.
17. Baumgartner BL, Bennett MR, Ferry M, Johnson TL, Tsimring LS, Hasty J. Antagonistic gene transcripts regulate adaptation to new growth environments. *Proceedings of the National Academy of Sciences*. 2011; 108:21087–21092.
18. Groisman A, Lobo C, Cho H, Campbell JK, Dufour YS, Stevens AM, Levchenko A. A microfluidic chemostat for experiments with bacterial and yeast cells. *Nat Methods*. 2005; 2:685–689. [PubMed: 16118639]
19. Wang CJ, Bergmann A, Lin B, Kim K, Levchenko A. Diverse sensitivity thresholds in dynamic signaling responses by social amoebae. *Sci Signal*. 2012; 5:ra17–ra17. [PubMed: 22375055]
20. Grilly C, Stricker J, Pang WL, Bennett MR, Hasty J. A synthetic gene network for tuning protein degradation in *Saccharomyces cerevisiae*. *Mol Syst Biol*. 2007; 3:127. [PubMed: 17667949]
21. Bennett MR, Pang WL, Ostroff NA, Baumgartner BL, Nayak S, Tsimring LS, Hasty J. Metabolic gene regulation in a dynamically changing environment. *Nature*. 2008; 454:1119–1122. [PubMed: 18668041]
22. Ronen M, Botstein D. Transcriptional response of steady-state yeast cultures to transient perturbations in carbon source. *Proc Natl Acad Sci US A*. 2006; 103:389–394.

23. McIsaac RS, Petti AA, Bussemaker HJ, Botstein D. Perturbation-based analysis and modeling of combinatorial regulation in the yeast sulfur assimilation pathway. *Mol Biol Cell*. 2012; 23:2993–3007. [PubMed: 22696683]
- **24. Hao N, O'Shea EK. Signal-dependent dynamics of transcription factor translocation controls gene expression. *Nat Struct Mol Biol*. 2012; 19:31–39. This study demonstrates that the *S. cerevisiae* TF Msn2 translocates to the nucleus with different dynamics depending on the nature and magnitude of the stress. 1-NM-PP1 is also used to program artificial Msn2 dynamics and show that promoters respond differently to these dynamics. [PubMed: 22179789]
- *25. Hao N, Budnik BA, Gunawardena J, O'Shea EK. Tunable signal processing through modular control of transcription factor translocation. *Science*. 2013; 339:460–464. In this follow up to the Hao 2012 study, the group uses dynamical 1-NM-PP1 signals, mathematical modeling, and Msn2 phosphorylation mutations to reveal that regulation of both nuclear import and export can drive translocating TFs to respond to dynamical input signals in different ways. [PubMed: 23349292]
26. Pierre-Jerome E, Jang SS, Havens KA, Nemhauser JL, Klavins E. Recapitulation of the forward nuclear auxin response pathway in yeast. *Proceedings of the National Academy of Sciences*. 2014; 111:9407–9412.
27. Muzzey D, Gómez-Urbe CA, Mettetal JT, van Oudenaarden A. A systems-level analysis of perfect adaptation in yeast osmoregulation. *Cell*. 2009; 138:160–171. [PubMed: 19596242]
28. Mettetal JT, Muzzey D, Gómez-Urbe C, van Oudenaarden A. The frequency dependence of osmo-adaptation in *Saccharomyces cerevisiae*. *Science*. 2008; 319:482–484. [PubMed: 18218902]
29. Hersen P, McClean MN, Mahadevan L, Ramanathan S. Signal processing by the HOG MAP kinase pathway. *Proceedings of the National Academy of Sciences*. 2008; 105:7165–7170.
30. Shimizu TS, Tu Y, Berg HC. A modular gradient-sensing network for chemotaxis in *Escherichia coli* revealed by responses to time-varying stimuli. *Mol Syst Biol*. 2010; 6:382. [PubMed: 20571531]
31. Doncic A, Skotheim JM. Feedforward regulation ensures stability and rapid reversibility of a cellular state. *Mol Cell*. 2013; 50:856–868. [PubMed: 23685071]
32. Doncic A, Falleur-Fettig M, Skotheim JM. Distinct interactions select and maintain a specific cell fate. *Mol Cell*. 2011; 43:528–539. [PubMed: 21855793]
33. Taylor RJ, Falconnet D, Niemistö A, Ramsey SA, Prinz S, Shmulevich I, Galitski T, Hansen CL. Dynamic analysis of MAPK signaling using a high-throughput microfluidic single-cell imaging platform. *Proceedings of the National Academy of Sciences*. 2009; 106:3758–3763.
34. Pelet S, Rudolf F, Nadal-Ribelles M, de Nadal E, Posas F, Peter M. Transient activation of the HOG MAPK pathway regulates bimodal gene expression. *Science*. 2011; 332:732–735. [PubMed: 21551064]
35. Fedor MJ, Kornberg RD. Upstream activation sequence-dependent alteration of chromatin structure and transcription activation of the yeast GAL1-GAL10 genes. *Mol Cell Biol*. 1989; 9:1721–1732. [PubMed: 2657404]
36. Razinkov IA, Baumgartner BL, Bennett MR, Tsimring LS, Hasty J. Measuring Competitive Fitness in Dynamic Environments. *J Phys Chem B*. 2013; 117:13175–13181. [PubMed: 23841812]
37. Çatay T, Turcotte M, Elowitz MB, Garcia-Ojalvo J, Süel GM. Architecture-dependent noise discriminates functionally analogous differentiation circuits. *Cell*. 2009; 139:512–522. [PubMed: 19853288]
38. Kuchina A, Espinar L, Çatay T, Balbin AO, Zhang F, Alvarado A, Garcia-Ojalvo J, Süel GM. Temporal competition between differentiation programs determines cell fate choice. *Mol Syst Biol*. 2011; 7:557. [PubMed: 22146301]
- **39. Vishnoi M, Narula J, Devi SN, Dao H-A, Igoshin OA, Fujita M. Triggering sporulation in *Bacillus subtilis* with artificial two-component systems reveals the importance of proper Spo0A activation dynamics. *Mol Microbiol*. 2013; 90:181–194. By placing the master *B. subtilis* sporulation factor Spo0A and its activating kinase KinC under different inducible promoters, the authors show that Spo0A activity must increase gradually for sporulation to occur properly, due in part to a requirement for temporally overlapping genes in chromosome segregation. [PubMed: 23927765]

40. Temme K, Salis H, Tullman-Ercek D, Levskaya A, Hong S-H, Voigt CA. Induction and relaxation dynamics of the regulatory network controlling the type III secretion system encoded within *Salmonella* pathogenicity island 1. *J Mol Biol.* 2008; 377:47–61. [PubMed: 18242639]
41. Fujita M, Losick R. Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A. *Genes & Development.* 2005; 19:2236–2244. [PubMed: 16166384]
42. Narula J, Devi SN, Fujita M, Igoshin OA. Ultrasensitivity of the *Bacillus subtilis* sporulation decision. *Proc Natl Acad Sci US A.* 2012; 109:E3513–22.
43. Svenningsen SL, Waters CM, Bassler BL. A negative feedback loop involving small RNAs accelerates *Vibrio cholerae*'s transition out of quorum-sensing mode. *Genes & Development.* 2008; 22:226–238. [PubMed: 18198339]
44. Locke JCW, Young JW, Fontes M, Hernández Jiménez MJ, Elowitz MB. Stochastic pulse regulation in bacterial stress response. *Science.* 2011; 334:366–369. [PubMed: 21979936]
45. Koirala S, Mears P, Sim M, Golding I, Chemla YR, Aldridge PD, Rao CV. A nutrient-tunable bistable switch controls motility in *Salmonella enterica* serovar Typhimurium. *MBio.* 2014; 5:e01611–14. [PubMed: 25161191]
46. Bashor CJ, Horwitz AA, Peisajovich SG, Lim WA. Rewiring Cells: Synthetic Biology as a Tool to Interrogate the Organizational Principles of Living Systems. *Annu Rev Biophys.* 2010; 39:515–537. [PubMed: 20192780]
47. Modell JW, Kambara TK, Perchuk BS, Laub MT. A DNA damage-induced, SOS-independent checkpoint regulates cell division in *Caulobacter crescentus*. *PLoS Biol.* 2014; 12:e1001977. [PubMed: 25350732]
48. Hoch JA. Regulation of the phosphorelay and the initiation of sporulation in *Bacillus subtilis*. *Annual review of microbiology.* 1993; 47:441–465.
49. Grossman AD. Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. *Annu Rev Genet.* 1995; 29:477–508. [PubMed: 8825484]
50. Veening JW, Murray H, Errington J. A mechanism for cell cycle regulation of sporulation initiation in *Bacillus subtilis*. *Genes & Development.* 2009; 23:1959–1970. [PubMed: 19684115]
- *51. Levine JH, Fontes ME, Dworkin J, Elowitz MB. Pulsed feedback defers cellular differentiation. *PLoS Biol.* 2012; 10:e1001252. The authors describe a positive feedback-based gene circuit architecture that gives rise to robust Spo0A~P pulses that defer sporulation for multiple cell cycles after starvation. This study highlights the need to generate artificial Spo0A~P dynamics to study the role of Spo0A~P pulsing in this complex network. [PubMed: 22303282]
52. Menolascina F, Fiore G, Orabona E, De Stefano L, Ferry M, Hasty J, di Bernardo M, di Bernardo D. In-Vivo Real-Time Control of Protein Expression from Endogenous and Synthetic Gene Networks. *PLoS Comput Biol.* 2014; 10:e1003625. [PubMed: 24831205]
- *53. Uhlenendorf J, Miermont A, Delaveau T, Charvin G, Fages F, Bottani S, Batt G, Hersen P. Long-term model predictive control of gene expression at the population and single-cell levels. *Proc Natl Acad Sci US A.* 2012; 109:14271–14276. Here, artificial gene expression dynamics are programmed in *S. cerevisiae* at the single-cell and population levels using a custom microfluidic device, sorbitol signals, the Hog pathway, fluorescence microscopy, and a computer-based feedback control system.
- **54. Hansen AS, O'Shea EK. Promoter decoding of transcription factor dynamics involves a trade-off between noise and control of gene expression. *Mol Syst Biol.* 2013; 9:704–704. In a second follow up to the Hao 2012 study, the authors use a multiplexed microfluidic device to create artificial Msn2 localization dynamics. Mathematical modeling and experiments are combined to show how TF dynamics can be differentially interpreted by promoters with different binding affinities and activation kinetics, and that oscillatory TF dynamics and slow promoter kinetics lead to higher gene expression noise. [PubMed: 24189399]
- **55. Olson EJ, Hartsough LA, Landry BP, Shroff R, Tabor JJ. Characterizing bacterial gene circuit dynamics with optically programmed gene expression signals. *Nat Methods.* 2014; 11:449–455. Light switchable TCSs, a batch culture optogenetic instrument, mathematical models, and a computational optimization algorithm are combined to program *E. coli* gene expression dynamics with exceptional control. The method is used to generate linear ramps and oscillations in TetR

expression to analyze the input/output dynamics of a TetR repressible promoter. The study uses step inputs to achieve predictable understanding of TCS dynamics. [PubMed: 24608181]

56. Miliadis-Argeitis A, Summers S, Stewart-Ornstein J, Zuleta I, Pincus D, El-Samad H, Khammash M, Lygeros J. In silico feedback for in vivo regulation of a gene expression circuit. *Nat Biotechnol.* 2011; 29:1114–1116. [PubMed: 22057053]
- *57. Melendez J, Patel M, Oakes BL, Xu P, Morton P, McClean MN. Real-time optogenetic control of intracellular protein concentration in microbial cell cultures. *Integr Biol (Camb).* 2014; 6:366–372. This study demonstrates optogenetic control of gene expression in a chemostat, which should be a major enabling approach going forward. [PubMed: 24477515]
58. Jayanthi S, Nilgiriwala KS, Del Vecchio D. Retroactivity controls the temporal dynamics of gene transcription. *ACS Synth Biol.* 2013; 2:431–441. [PubMed: 23654274]
59. Jiang P, Ventura AC, Sontag ED, Merajver SD, Ninfa AJ, Del Vecchio D. Load-induced modulation of signal transduction networks. *Sci Signal.* 2011; 4:ra67–ra67. [PubMed: 21990429]
60. Cardinale S, Arkin AP. Contextualizing context for synthetic biology – identifying causes of failure of synthetic biological systems. *Biotechnology Journal.* 2012; 7:856–866. [PubMed: 22649052]
61. Cookson NA, Mather WH, Danino T, Mondragón-Palomino O, Williams RJ, Tsimring LS, Hasty J. Queueing up for enzymatic processing: correlated signaling through coupled degradation. *Mol Syst Biol.* 2011; 7:561–561. [PubMed: 22186735]
62. Prindle A, Selimkhanov J, Li H, Razinkov I, Tsimring LS, Hasty J. Rapid and tunable post-translational coupling of genetic circuits. *Nature.* 2014; 508:387–391. [PubMed: 24717442]
63. Tan C, Marguet P, You L. Emergent bistability by a growth-modulating positive feedback circuit. *Nature Chemical Biology.* 2009; 5:842–848.
64. Tabor JJ, Bayer TS, Simpson ZB, Levy M, Ellington AD. Engineering stochasticity in gene expression. *Mol Biosyst.* 2008; 4:754–761. [PubMed: 18563250]
65. Brewster RC, Weinert FM, Garcia HG, Song D, Rydenfelt M, Phillips R. The transcription factor titration effect dictates level of gene expression. *Cell.* 2014; 156:1312–1323. [PubMed: 24612990]
66. Cardinale S, Joachimiak MP, Arkin AP. Effects of genetic variation on the E. coli host-circuit interface. *Cell Rep.* 2013; 4:231–237. [PubMed: 23871664]
67. Arkin AP. A wise consistency: engineering biology for conformity, reliability, predictability. *Curr Opin Chem Biol.* 2013; 17:893–901. [PubMed: 24268562]
68. Del Vecchio D, Ninfa AJ, Sontag ED. Modular cell biology: retroactivity and insulation. *Mol Syst Biol.* 2008; 4:161. [PubMed: 18277378]
69. Pelet S, Rudolf F, Nadal-Ribelles M, de Nadal E, Posas F, Peter M. Transient activation of the HOG MAPK pathway regulates bimodal gene expression. *Science.* 2011; 332:732–735. [PubMed: 21551064]
70. Lipan O, Wong WH. The use of oscillatory signals in the study of genetic networks. *Proc Natl Acad Sci US A.* 2005; 102:7063–7068.
71. Ang J, Ingalls B, McMillen D. Probing the input-output behavior of biochemical and genetic systems system identification methods from control theory. *Meth Enzymol.* 2011; 487:279–317. [PubMed: 21187229]
72. Paliwal S, Wang CJ, Levchenko A. Pulsing cells: how fast is too fast? *HFSP J.* 2008; 2:251–256. [PubMed: 19404435]
73. Wei P, Wong WW, Park JS, Corcoran EE, Peisajovich SG, Onuffer JJ, Weiss A, Lim WA. Bacterial virulence proteins as tools to rewire kinase pathways in yeast and immune cells. *Nature.* 2012; 488:384–388. [PubMed: 22820255]
74. Schaber J, Baltanas R, Bush A, Klipp E, Colman-Lerner A. Modelling reveals novel roles of two parallel signalling pathways and homeostatic feedbacks in yeast. *Mol Syst Biol.* 2012; 8:622. [PubMed: 23149687]
75. Olson EJ, Tabor JJ. Post-translational tools expand the scope of synthetic biology. *Curr Opin Chem Biol.* 2012; 16:300–306. [PubMed: 22766485]
76. Khlebnikov A, Risa Ø, Skaug T, Carrier TA, Keasling JD. Regulatable arabinose-inducible gene expression system with consistent control in all cells of a culture. *J Bacteriol.* 2000; 182:7029–7034. [PubMed: 11092865]

77. Hawkins KM, Smolke CD. The regulatory roles of the galactose permease and kinase in the induction response of the GAL network in *Saccharomyces cerevisiae*. *J Biol Chem*. 2006; 281:13485–13492. [PubMed: 16524886]
78. Brautaset T, Lale R, Valla S. Positively regulated bacterial expression systems. *Microb Biotechnol*. 2009; 2:15–30. [PubMed: 21261879]
- *79. Toettcher JE, Weiner OD, Lim WA. Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. *Cell*. 2013; 155:1422–1434. Though a mammalian signaling pathway was analyzed, this work is an outstanding demonstration of how optogenetics and dynamical perturbations can avoid pitfalls of chemical effectors and be used to unravel how signals flow through a highly complex gene regulatory network. [PubMed: 24315106]
80. Cox RS, Surette MG, Elowitz MB. Programming gene expression with combinatorial promoters. *Mol Syst Biol*. 2007; 3:145. [PubMed: 18004278]
81. Vavrová L, Muchová K, Barák I. Comparison of different *Bacillus subtilis* expression systems. *Res Microbiol*. 2010; 161:791–797. [PubMed: 20863884]
82. Redden H, Morse N, Alper HS. The synthetic biology toolbox for tuning gene expression in yeast. *FEMS Yeast Res*. 2014; 10.1111/1567-1364.12188.
83. Lutz R, Bujard H. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res*. 1997; 25:1203–1210. [PubMed: 9092630]
84. de Boer HA, Comstock LJ, Vasser M. The tac promoter: a functional hybrid derived from the trp and lac promoters. *Proc Natl Acad Sci US A*. 1983; 80:21–25.
85. Tabor, JJ.; Groban, ES.; Voigt, CA. *Systems Biology and Biotechnology of Escherichia coli*. Springer; Netherlands: 2009. Performance Characteristics for Sensors and Circuits Used to Program *E. coli*; p. 401-439.
86. Möglich A, Ayers RA, Moffat K. Design and Signaling Mechanism of Light-Regulated Histidine Kinases. *J Mol Biol*. 2009; 385:1433–1444. [PubMed: 19109976]
87. Ohlendorf R, Vidavski RR, Eldar A, Moffat K, Möglich A. From Dusk till Dawn: One-Plasmid Systems for Light-Regulated Gene Expression. *J Mol Biol*. 2012; 416:534–542. [PubMed: 22245580]
88. Tabor JJ, Levskaya A, Voigt CA. Multichromatic control of gene expression in *Escherichia coli*. *J Mol Biol*. 2011; 405:315–324. [PubMed: 21035461]
89. Levskaya A, Chevalier AA, Tabor JJ, Simpson ZB, Lavery LA, Levy M, Davidson EA, Scouras A, Ellington AD, Marcotte EM, et al. Synthetic biology: engineering *Escherichia coli* to see light. *Nature*. 2005; 438:441–442. [PubMed: 16306980]
90. Tabor JJ, Salis HM, Simpson ZB, Chevalier AA, Levskaya A, Marcotte EM, Voigt CA, Ellington AD. A synthetic genetic edge detection program. *Cell*. 2009; 137:1272–1281. [PubMed: 19563759]
91. Ryu M-H, Gomelsky M. Near-infrared Light Responsive Synthetic c-di-GMP Module for Optogenetic Applications. *ACS Synth Biol*. 2014; 10.1021/sb400182x.
- *92. Schmidl SR, Sheth RU, Wu A, Tabor JJ. Refactoring and optimization of light-switchable *Escherichia coli* two-component systems. *ACS Synth Biol*. 2014; 3(11):820–31. The light-switchable TCSs from the Olson 2014 study are systematically optimized, resulting in a number of improved features including up to 117-fold dynamic range and less cross-reactivity and strain-dependence. These v2.0 systems will allow more gene networks to be characterized in more bacteria over a wider range of expression levels of the components. [PubMed: 25250630]
93. Rizzini L, Favory J-J, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schäfer E, Nagy F, Jenkins GI, et al. Perception of UV-B by the *Arabidopsis* UVR8 protein. *Science*. 2011; 332:103–106. [PubMed: 21454788]
94. Pathak GP, Strickland D, Vrana JD, Tucker CL. Benchmarking of optical dimerizer systems. *ACS Synth Biol*. 2014; 3:832–838. [PubMed: 25350266]
95. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL. Rapid blue-light-mediated induction of protein interactions in living cells. *Nat Methods*. 2010; 7:973–975. [PubMed: 21037589]

96. Shimizu-Sato S, Huq E, Tepperman JM, Quail PH. A light-switchable gene promoter system. *Nat Biotechnol.* 2002; 20:1041–1044. [PubMed: 12219076]
97. Miyake K, Abe K, Ferri S, Nakajima M, Nakamura M, Yoshida W, Kojima K, Ikebukuro K, Sode K. A green-light inducible lytic system for cyanobacterial cells. *Biotechnology for Biofuels.* 2014; 7:56. [PubMed: 24713090]
98. Hurley JM, Chen C-H, Loros JJ, Dunlap JC. Light-inducible system for tunable protein expression in *Neurospora crassa*. *G3 (Bethesda).* 2012; 2:1207–1212. [PubMed: 23050231]
99. Renicke C, Schuster D, Usherenko S, Essen L-O, Taxis C. A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem Biol.* 2013; 20:619–626. [PubMed: 23601651]
100. Usherenko S, Stibbe H, Muscò M, Essen L-O, Kostina EA, Taxis C. Photo-sensitive degen variants for tuning protein stability by light. *BMC Syst Biol.* 2014; 8:128. [PubMed: 25403319]
- *101. Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, Di Ventura B. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. *Nat Commun.* 2014; 5:4404. An optogenetic tool for blue light/dark reversible nuclear translocation of proteins in yeast. This tool could be combined with constitutively active TFs and batch, continuous culture or microscopic illumination methods to investigate how different translocation dynamics regulate different promoters across the genome or in single cells. [PubMed: 25019686]
102. Richter F, Scheib US, Mehlhorn J, Schubert R, Wietek J, Gernetzki O, Hegemann P, Mathes T, Möglich A. Upgrading a microplate reader for photobiology and all-optical experiments. *Photochem Photobiol Sci.* 2014; 10.1039/c4pp00361f.
103. Davidson EA, Basu AS, Bayer TS. Programming microbes using pulse width modulation of optical signals. *J Mol Biol.* 2013; 425:4161–4166. [PubMed: 23928560]
- **104. Takahashi CN, Miller AW, Ekness F, Dunham MJ, Klavins E. A Low Cost, Customizable Turbidostat for Use in Synthetic Circuit Characterization. *ACS Synth Biol.* 2014; 34:15–22. An open-source, 8-chamber turbidostat with automated media source switching and GFP detection capabilities is described. The authors demonstrate the device by studying the auxin plant hormone pathway in *S. cerevisiae* under different cell densities, and by dynamically inducing GFP expression from an IPTG-inducible *E. coli* strain over 40 hours.
105. Bennett MR, Hasty J. Microfluidic devices for measuring gene network dynamics in single cells. *Nat Rev Genet.* 2009; 10:628–638. [PubMed: 19668248]
106. Lin B, Levchenko A. Microfluidic technologies for studying synthetic circuits. *Curr Opin Chem Biol.* 2012; 16:307–317. [PubMed: 22609335]
107. Hsiao V, de Los Santos ELC, Whitaker WR, Dueber JE, Murray RM. Design and Implementation of a Biomolecular Concentration Tracker. *ACS Synth Biol.* 2014; 10.1021/sb500024b.
108. Vardi N, Levy S, Gurvich Y, Polacheck T, Carmi M, Jaitin D, Amit I, Barkai N. Sequential feedback induction stabilizes the phosphate starvation response in budding yeast. *Cell Rep.* 2014; 9:1122–1134. [PubMed: 25437565]
109. Havens KA, Guseman JM, Jang SS, Pierre-Jerome E, Bolten N, Klavins E, Nemhauser JL. A synthetic approach reveals extensive tunability of auxin signaling. *Plant Physiol.* 2012; 160:135–142. [PubMed: 22843664]
110. Crane MM, Clark IBN, Bakker E, Smith S, Swain PS. A microfluidic system for studying ageing and dynamic single-cell responses in budding yeast. *PLoS ONE.* 2014; 9:e100042. [PubMed: 24950344]
111. Görner W, Durchschlag E, Martínez-Pastor MT, Estruch F, Ammerer G, Hamilton B, Ruis H, Schüller C. Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes & Development.* 1998; 12:586–597. [PubMed: 9472026]
112. Garmendia-Torres C, Goldbeter A, Jacquet M. Nucleocytoplasmic oscillations of the yeast transcription factor Msn2: evidence for periodic PKA activation. *Curr Biol.* 2007; 17:1044–1049. [PubMed: 17570669]
113. Martínez-Pastor MT, Marchler G, Schüller C, Marchler-Bauer A, Ruis H, Estruch F. The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional

- induction through the stress response element (STRE). *EMBO J.* 1996; 15:2227–2235. [PubMed: 8641288]
114. Cai L, Dalal CK, Elowitz MB. Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature.* 2008; 455:485–490. [PubMed: 18818649]
 - *115. Cai H, Katoh-Kurasawa M, Muramoto T, Santhanam B, Long Y, Li L, Ueda M, Iglesias PA, Shaulsky G, Devreotes PN. Nucleocytoplasmic shuttling of a GATA transcription factor functions as a development timer. *Science.* 2014; 343:1249531–1249531. This study shows that extracellular cAMP pulses result in dynamical nuclear shutting of a key TF thereby inducing promoters and controlling the single to multicellular transition in *Dictyostelium*. The authors suggest this pathway is a timing mechanism for coordinating differentiation decisions amongst multiple individuals in an environment. [PubMed: 24653039]
 116. Dénervaud N, Becker J, Delgado-Gonzalo R, Damay P, Rajkumar AS, Unser M, Shore D, Naef F, Maerkl SJ. A chemostat array enables the spatio-temporal analysis of the yeast proteome. *Proc Natl Acad Sci US A.* 2013; 110:15842–15847.
 117. Dalal CK, Cai L, Lin Y, Rahbar K, Elowitz MB. Pulsatile Dynamics in the Yeast Proteome. *Curr Biol.* 2014; 1016/j.cub.2014.07.076.
 118. Levskaya A, Weiner OD, Lim WA, Voigt CA. Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature.* 2009; 461:997–1001. [PubMed: 19749742]
 119. Toettcher JE, Gong D, Lim WA, Weiner OD. Light-based feedback for controlling intracellular signaling dynamics. *Nat Methods.* 2011; 8:837–839. [PubMed: 21909100]
 - *120. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell.* 2013; 152:1173–1183. This paper describes a now widely used method for repressing transcription of any *E. coli* gene using single guide RNAs and dCas9. This method could be combined with the optogenetic method of Olson and coworkers to dynamically control expression of any gene in the *E. coli* genome and along with FP and omics measurements, characterize the dynamics of the greater *E. coli* transcriptional regulatory network. [PubMed: 23452860]
 - *121. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell.* 2013; 154:442–451. The CRISPR interference pathway can be used to repress transcription of native genes in yeast, which can likely be used for dynamical characterization of native regulatory networks as in bacteria. [PubMed: 23849981]
 122. Bikard D, Jiang W, Samai P, Hochschild A, Zhang F, Marraffini LA. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. *Nucleic Acids Res.* 2013; 41:7429–7437. [PubMed: 23761437]
 123. Farzadfard F, Perli SD, Lu TK. Tunable and multifunctional eukaryotic transcription factors based on CRISPR/Cas. *ACS Synth Biol.* 2013 [no volume].
 124. Hirose Y, Shimada T, Narikawa R, Katayama M, Ikeuchi M. Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. *Proc Natl Acad Sci US A.* 2008; 105:9528–9533.
 125. Brewster JL, Gustin MC. Hog1: 20 years of discovery and impact. *Sci Signal.* 2014; 7:re7–re7. [PubMed: 25227612]

Highlights

Gene regulation is highly dynamic, but most experimental perturbations are static

Engineering approaches are being used to characterize gene network dynamics

Recent optogenetic methods and instrumentation allow more advanced dynamical studies

The role of transcription factor pulsing in sporulation and other pathways could be revealed

Figure 1.

Characterizing gene networks with dynamical extracellular and intracellular perturbations.

a) Simplified diagram of the *S. cerevisiae* glucose-galactose switch. Glucose-induced degradation of a subset of *GAL* mRNAs is depicted in orange. b) Schematic of 'Dial-a-Wave' [21,105] microfluidic device enabling independent dynamical control of the amount of glucose and galactose in the media. c) When glucose is varied sinusoidally at low frequency (period greater than 67.5 minutes) and galactose is held constant (top), *GAL1* expression is sinusoidal (bottom left). Model simulations of *GAL1* response with and without glucose-inducible mRNA degradation (bottom right). Figure adapted from [21]. d) Schematic of the synthetic *B. subtilis* sporulation system with *kinC* and *spo0A* under chemically-inducible promoters [39]. The Spo0A regulon includes low-threshold genes such as *RacA* (involved in chromosomal segregation) and *FtsZ* (necessary for cell division during sporulation), and high threshold genes such as *DivIVA* (also required for chromosomal segregation). e) When Spo0A~P is made to increase gradually, σ^H -induced *RacA* and *FtsZ* are expressed before *DivIVA* is repressed, allowing proper chromosome segregation and spore formation. f) Unnaturally fast Spo0A~P increases cause *DivIVA* to be repressed before *RacA* and *FtsZ* can be produced, resulting in improper chromosome partitioning prior to cell division, and defective spores. Figure adapted from [39].

Figure 2.

Engineering view of two microbial signal transduction pathways. a) The CcaS-CcaR green/red light-reversible bacterial two-component system [88,92,124]. The sensor histidine kinase CcaS (transducer) is produced in a green light sensitive, dark-adapted ground state (termed Pg) wherein it acts as a phosphatase against the phosphorylated form of the response regulator CcaR (CcaR~P). Absorption of a green photon flips CcaS Pg to a kinase active red light sensitive state (Pr) where it phosphorylates CcaR to CcaR~P. Absorption of a red photon flips CcaS Pr back to the Pg state. The phosphoryl flux (kinase activity toward CcaR minus phosphatase activity toward CcaR~P) is a biological signal. A specific phosphoryl flux results in a specific concentration of CcaR~P, a second biological signal. An inducible promoter, P_{cpcG2}, is induced by CcaR~P, thus converting the second biological signal into a transcription rate, a third biological signal which is the output of this system. b) The *S. cerevisiae* osmotic stress response pathway [27–29,125]. The effector signal (osmotic pressure) is detected by two signaling branches, Sln1 and Sho1, and converted into kinase activity toward Hog1. In the Sln1 branch, Sln1/Ypd1/Ssk1 constitute a phosphorelay-like subsystem that transduces the osmotic pressure into phosphorylation of the mitogen-activated protein kinase kinase kinases (MAPKKKs) Ssk2 and Ssk22. Sho1, Hkr, and Msb2 are proposed sensors of the Sho1 branch, and their output is phosphorylation of MAPKKK Ste11. Phosphorylated Ssk2, Ssk22 and Ste11, in turn, phosphorylate the MAPKK Pbs2, which then phosphorylates Hog1. Phosphorylated Hog1 enters the nucleus and phosphorylates/activates several TFs, activating target promoters whose output is transcription rate of stress and glycerol-producing genes.

Figure 3.

Hardware for making dynamical effector and biological signals. a) The Light Tube Array (LTA) (left). A computational algorithm utilizes simulations from the mathematical model of the CcaS-CcaR system (center) to calculate a time-varying light input (effector signal) that will result in a desired gene expression output (biological signal). This approach was used to create linear and sinusoidal TetR signals (reported by a *gfp* gene expressed from the same mRNA) and thereby characterize $P_{\text{LtetO-1}}$ input/output dynamics. mCherry was used to characterize $P_{\text{LtetO-1}}$ output. Figure adapted from [55]. b) The Flexostat (left) was used to characterize GFP expression from an IPTG-inducible (center) *E. coli* strain [104]. The device was programmed to automatically switch from media lacking IPTG to media containing IPTG every 7 hours, for 1 hour at a time. The resulting IPTG concentration in the chamber is changing dynamically for most of the experiment (not shown). Automatic on-line acquisition of fluorescence shows GFP response dynamics at high resolution (right). Figure adapted from [104]. c) The CellASIC ONIX microfluidic platform (left) was used to characterize the *B. subtilis* general stress response (center) when ethanol and salt stresses are applied at different rates. Figure adapted from [16].

Table 1

Different types of dynamic perturbation signals and their uses

Input Signal	Description	Variable Parameters	Information that can be obtained by measuring system output	Experimental Requirements
Constant Conditions	<ul style="list-style-type: none">A: Input Level	<ul style="list-style-type: none">Is the system at steady state, or is it dynamic even in a constant environment?If steady state, what is the dose-response curve? This includes minimum and maximum output, input level that gives half-maximum output (K), and apparent cooperativity (n).If dynamic, does the system oscillate, or stochastically pulse? Are the amplitude or frequency of the pulses modulated by the input? [1].	<ul style="list-style-type: none">Continuous culture for ideal constant conditions.Can be approximated in batch culture if properly controlled.
Step: Sudden perturbation	<ul style="list-style-type: none">L: Low inputH: High inputSign (+/-)	<ul style="list-style-type: none">Is there a delay before change in output? This can indicate an internal process that takes a fixed time, such as transcription, translation, or protein maturation.Does output reach steady state after the step? How quickly? No internal process can be slower than the overall system response.Does the output overshoot before reaching the new steady state? This indicates negative feedback [2].Does the output eventually return to the initial level (perfect adaptation)? This requires a negative feedback loop with an “integrator”, a component whose output is the integral of its input. For example, a saturated enzymatic reaction integrates the concentration of the enzymes [27].	<ul style="list-style-type: none">Chemical effectors: step up can be easily approximated in batch, step down requires washing.Light effectors: Steps in both directions can be easily performed even in batch.Continuous culture required to achieve ideal constant conditions before and after step.

Input Signal	Description	Variable Parameters	Information that can be obtained by measuring system output	Experimental Requirements
Ramp: Gradual shift from one input level to another	<ul style="list-style-type: none"> L: Low input H: High input d: duration n: ramp order (1: linear, 2: quadratic, etc.) 	<ul style="list-style-type: none"> Is the output sensitive to the rate of change of input? This may indicate a slow negative feedback loop [16] or a feedforward loop [2]. Does the output perfectly adapt during the ramp? Integral feedback systems with 2 or more integrators in series perfectly adapt to a linear ramp. Systems with 3 or more integrators perfectly adapt to a quadratic ramp, and so on [27]. 	<ul style="list-style-type: none"> Chemical effectors: Ramp up can be approximated in batch by using several smaller steps. Ramp down difficult without continuous culture device. Light effectors: ramps of any low, high, or duration can be done in both directions even in batch.
Oscillatory wave: Periodic shifts between two different input levels	<ul style="list-style-type: none"> A: Amplitude P: Period, inverse of frequency O: Offset A: Pulse amplitude d: Pulse duration 	<ul style="list-style-type: none"> What is the highest frequency input to which a system can respond? This frequency is the system's bandwidth. Reactions in a system must occur at least as quickly as the bandwidth [70,71]. What are the dominant (i.e. rate-limiting) processes of the system? These are the ones that operate on a timescale close to the bandwidth. A simple linear model can be fit to the frequency response, which can shed light into the number and identity of dominant processes. This has been used to decompose the yeast osmotic shock response into Hog1-dependent and independent components [28]. 	<ul style="list-style-type: none"> Chemical effectors: Requires continuous culture device or microfluidics. Light effectors: can be done even in batch.