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Chapter

Synthetic Gene Circuits for Antimicrobial Resistance and Cancer Research

Kevin S. Farquhar, Michael Tyler Guinn, Gábor Balázsi and Daniel A. Charlebois

Abstract

Mathematical models and synthetic gene circuits are powerful tools to develop novel treatments for patients with drug-resistant infections and cancers. Mathematical modeling guides the rational design of synthetic gene circuits. These systems are then assembled into unified constructs from existing and/or modified genetic components from a range of organisms. In this chapter, we describe modeling tools for the design and characterization of chemical- and light-inducible synthetic gene circuits in different organisms and highlight how synthetic gene circuits are advancing biomedical research. Specifically, we demonstrate how these quantitative model systems are being used to study drug resistance in microbes and to probe the spatial–temporal dimensions of cancer in mammalian cells.

Keywords: antimicrobial resistance, synthetic gene circuits, mathematical models, optogenetics, cancer

1. Introduction

A primary goal of synthetic biology is to rationally design and engineer synthetic gene circuits as tools to advance basic research [1, 2], optimize the production of chemicals or biofuels [3, 4], build biocomputational systems [5], and enhance clinical therapeutics [6]. Control of synthetic gene circuits at the transcriptional level (transcription is the process of transcribing mRNA from a DNA template) has been demonstrated through chemical- and light-based stimuli [7, 8]. The transcriptional network architecture (how genes are connected to and regulate each other through transcription factor proteins) affects the properties of gene expression, in terms of average expression levels as well as the degree of expression variability inside a single cell or across a cell population [9]. Throughout this chapter, we will use the term "synthetic gene circuits" to describe synthetic systems and the term "gene networks" to described natural systems.

Fluctuations in the biochemical processes of transcription and translation (translation is the process of translating amino acid-based proteins from a nucleotide-based mRNA template) are referred to as gene expression noise [10]. Gene expression noise leads to heterogeneity among genetically identical cells in the same environment and can affect the survival of microorganisms [11]. For instance,

gene expression noise has been shown to promote drug resistance in microbes [12]. Similarly, gene expression noise is thought to play an important role in tumorigenesis and the development of resistance during cancer chemotherapy [13]. Mathematical models and synthetic gene circuits have established that the architecture of the gene network modulates gene expression noise [14].

Electronic circuits inspired the development of synthetic gene circuits, with mathematical representations of natural and synthetic networks successfully predicting their effects on gene expression [15]. A milestone study in *E. coli* demonstrated that negative feedback stabilizes the gene circuit's response to expression fluctuations [16]. The first synthetic toggle switch circuit in *E. coli* mimicked the electronic version and served as a simplified version of the naturally occurring bacteriophage lambda switch [17, 18]. The construction of a synthetic biological clock in *E. coli* permitted oscillations in gene expression to be tuned to a particular frequency [19]. By mimicking natural gene networks, synthetic gene circuits generate insights on how complex biological systems work by breaking down natural networks into their components, which is highly beneficial in basic biomedical research [20].

Optogenetics is the control of cellular components using electromagnetic radiation. Like other synthetic systems, optogenetic components can be engineered into gene circuits to precisely control cellular processes such as gene expression or protein activity in prokaryotic and eukaryotic cells; the performance of optogenetic gene circuits can be optimized in an iterative model-experiment cycle. However, unlike previous gene circuits, optogenetics offers the ability to control gene expression at a single-cell resolution. The fast temporal and single-cell spatial resolutions that light provides as a stimulus for gene circuits is unmatched; chemical stimulus regulates transcription on longer timescales and at a cell-population level. Like their gene circuit predecessors, optogenetic gene circuits can be used to control functional proteins. Optogenetic tools are especially suited to investigate gene function at the single-cell level. For instance, researchers can take a gene of interest, such as KRAS which is often found mutated in cancers [21], and integrate it into an optogenetic gene circuit to explore the transcriptional and translational effects on cellular phenotypes by stimulating individual cells with visible light. It is worth noting that although in this chapter we focus on optogenetic applications involving visible light, some optogenetic tools have been developed using other regions of the electromagnetic spectrum, including near-infrared [22] and UV [23] radiation.

This chapter describes the construction and characterization of synthetic gene circuits in yeast and mammalian cells (Section 2) and optogenetic gene circuits in mammalian cells (Section 3) with various transcriptional network architectures, along with their applications in biomedical research. The mathematical approaches to model synthetic and optogenetic gene circuits are also discussed.

2. Synthetic gene circuits

2.1 Positive feedback gene circuits in yeast

A positive feedback synthetic gene circuit was first constructed in yeast to convert a continuous gradient of a constitutively expressed transcriptional activator into a cell phenotype switch, resembling analog to digital signal conversion [24]. Subsequently, a positive feedback (PF) gene circuit was genomically integrated into
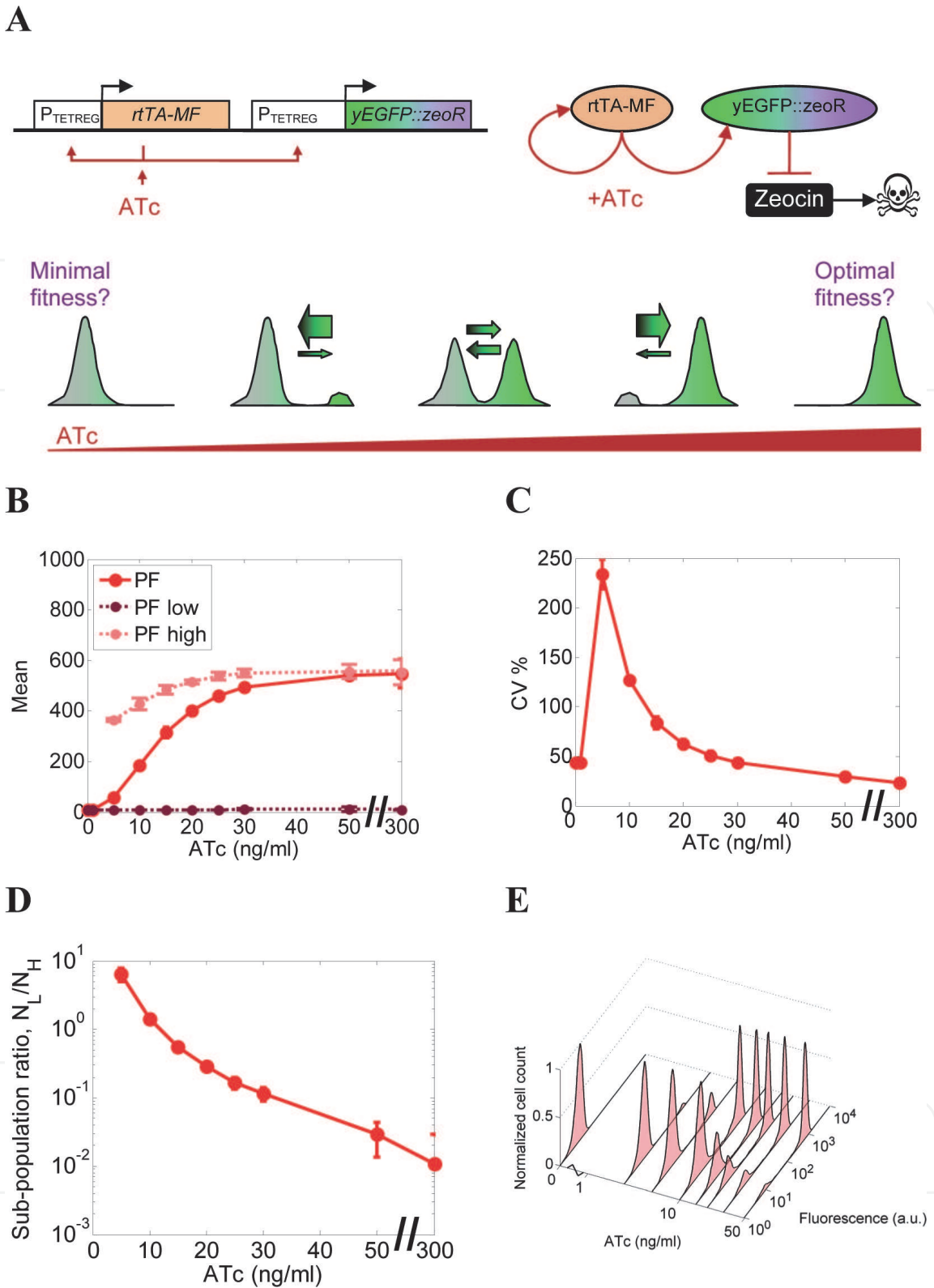
Figure 1.
*(A) Schematic of the positive feedback (PF) synthetic gene circuit in yeast (top left). The regulator rtTA is toxic when active, but the gene circuit prevents Zeocin toxicity by activating the ZeoR gene (top right). The role of cellular memory in optimizing fitness is shown in the bottom panel. (B-E) dose responses. (B) Mean expression levels of the PF gene circuit and of the cell sorted low- and high-expression states. (C) Gene expression noise as determined from the coefficient of variation (CV). (D) Subpopulation ratio of low-expressing cells to high-expressing cells. (E) Steady-state gene expression distributions at a single-cell resolution. From **Figure 2** "characterization of gene expression in cells bearing PF circuit" by Nevozhay, D. and Adams, R. et al. in [25] located at https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002480 under a CC BY 4.0 license with panel label font modified.*

the budding yeast *Saccharomyces cerevisiae* to investigate how cell population fitness (growth rate) and subpopulations emerge from the molecular-level kinetics of gene networks and single-cell division rates [25]. Synthetic gene circuits with positive feedback and cooperativity can display bistability, where cells switch between two gene expression states with a cellular "memory" that corresponds to the temporal maintenance of each state [26, 27].

In the PF gene circuit, the regulator reverse tetracycline-controlled trans-activator (rtTA) binds to its own promoter in the presence of tetracyclines (**Figure 1A**, top left) [28]. The genetic engineering approaches mirrored the assembly of a negative feedback (NF) circuit in yeast [29]. Unlike the NF circuit, toxicity exists after activating the regulator gene rtTA, which sequesters general transcription factors from vital cellular processes [30]. Additionally, the construct controlled the drug resistance gene ZeoR, which confers resistance to the antibiotic Zeocin (**Figure 1A**, top right).

The PF gene circuit exhibits a sigmoidal gene expression dose response at the population level (**Figure 1B**). The expression dose–responses for low- and high-expressing subpopulations were determined using a bimodality detection algorithm [28]. The gene expression noise level peaked at an intermediate inducer (anhydrotetracycline or ATc) concentration (**Figure 1C**). The shift in the gene expression distribution peaks over increasing inducer levels is reflected by the subpopulation ratio changes (**Figure 1D**). The gene expression distributions for the ATc dose response display bimodality (**Figure 1E**).

Testing multiple levels of inducer and drug determined a "fitness landscape" that quantitatively mapped population growth rates to unique combinations of ATc and Zeocin concentrations. The yeast PF system demonstrated the need to incorporate the cellular memory associated with gene expression states (**Figure 1A**, bottom) to computationally predict the fitness landscape [31]; the growth rate under Zeocin treatment was the highest at the minimal level of induction that lead to bimodal expression [28]. These computational predictions guided laboratory experiments to identify environmental conditions that defined a "sweet spot" of drug resistance, which balanced the costs of expressing rtTA with benefits of expressing ZeoR.

The yeast PF synthetic gene circuit was subsequently used to study evolutionary dynamics under various levels of induction and drug treatment [32]. This microbial evolution experiment included conditions at one edge or both edges (saturating molecular levels or none) of the fitness landscape, as well as at intermediate levels of induction with or without Zeocin. The evolved populations were frozen at specific time points and subsequently reestablished to test in various conditions compared to ancestral cells. Additionally, the evolution experiments were combined with simulations to predict the types of mutations that could arise under induction and drug treatment [32].

Full induction without drug treatment led to breakdown of the regulator rtTA over time through full and partial knock-out mutations, which improved fitness without Zeocin [32]. Yet, during follow-up evolution experiments in high induction with Zeocin partial rtTA knockout mutants regained function [31]. Full Zeocin treatment without induction eventually established populations with higher expression, potentially through mutations in the drug resistance gene and promoter linked with extra-circuit mutations. High induction with Zeocin led to accumulated mutations in rtTA, possibly lowering the effectiveness of the regulator as shown by decreased inducer sensitivity [32]. After intermediate induction with Zeocin, experiments on evolved populations during reintroduction of drug under intermediate induction uncovered the two expression distribution peaks shifting towards each other leading to a single fitness peak; this highlighted the role of noise in driving evolution through the trade-off between rtTA toxicity and drug resistance.
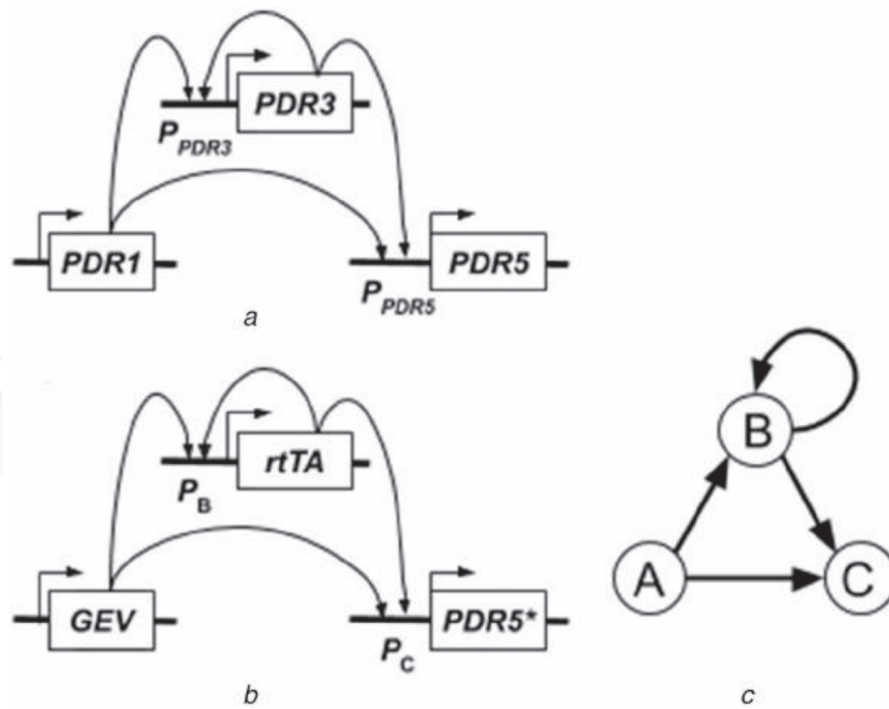
Figure 2.
*(a) Natural pleiotropic drug resistance (PDR) network in yeast. (b) Synthetic PDR gene circuit. (c) Core PDR network architecture. From **Figure 1** "network schematics" by Brendan Camellato et al. in [27] located at https://ietresearch.onlinelibrary.wiley.com/doi/10.1049/enb.2019.0009 under a CC BY-NC-ND 3.0 license.*

2.2 Experiments and computational models of positive feedback and feedforward circuits in yeast

Along with positive feedback and negative feedback circuit architectures, feedforward loop (FFL) architectures (a three-gene network composed of two input transcription factors, one of which regulates the other, both jointly regulating a target gene) may have evolved in natural gene regulatory networks to enhance fitness [26, 33]. In *S. cerevisiae*, the pleiotropic drug resistance (PDR) network contains a positive feedback loop embedded in a feedforward loop (FFL + PF) (**Figure 2a**) [34]. The PDR network provides multidrug resistance through an ABC transporter pump protein encoded by the PDR5 gene. A similar FFL + PF network may enhance drug resistance in human cancer cells [35].

Mathematical models of gene regulatory networks can predict biological responses, which is essential to optimally design synthetic gene circuits and to guide experiments. A minimal model of the PDR network found that the positive feedback and feedforward loop architectures sustain transcription and can stabilize expression of the network when the drug is transient or fluctuating [33]. The minimal model also predicted increased gene expression noise (in terms of increased noise magnitude and longer cellular memory timescales) in the FFL and FFL + PF networks. Overall, the FFL and FFL + PF network architectures were found to enhance drug resistance *in silico*.

The minimal model of the PDR network was described by the following system of coupled ordinary differential equations (ODEs) [33]:

$$\frac{dPDR3}{dt} = \alpha_{PDR3}\omega_1 f_{PDR3}(PDR1, PDR3) - PDR3 \qquad (1)$$

$$\frac{dPDR5}{dt} = \alpha_{PDR5} f_{PDR5}(PDR1, PDR3) - PDR5$$

where PDR1 was treated as an adjustable parameter. α_{PDR3} and α_{PDR5} are the maximum levels of activated protein production for the variables PDR3 and PDR5, respectively. The Boolean parameter ω_1 describes the activation of PDR3 by PDR1. Here the dilution and degradation rates of PDR3 and PDR5 were set to unity. The functions that describe how PDR3 and PDR5 are regulated are given by:

$$f_{PDR3}(PDR1, PDR3) = \frac{(PDR1 + \omega_2 PDR3)^n}{K^n + (PDR1 + \omega_2 PDR3)^n} \tag{2}$$

$$f_{PDR5}(PDR1, PDR3) = \frac{(PDR1 + PDR3)^n}{K^n + (PDR1 + PDR3)^n}$$

where, n and K are the Hill coefficient and half-maximal activation parameter, respectively. The Boolean parameter ω_2 describes the presence or absence of positive feedback regulation on PDR3. The minimal model and a more comprehensive model (presented below) were translated into biochemical reactions that were simulated using the Gillespie stochastic simulation algorithm [36, 37].

A more comprehensive model, known as the PDR5 transcriptional network model, incorporated the dynamics of the PDR5 efflux protein pump and the negative feedback produced when PDR5 eliminates the drug from the cell [33]. The PDR5 transcriptional network model can be described by the following system of coupled ODEs:

$$\frac{dPDR1}{dt} = \alpha_0 + \alpha_{PDR1} \frac{D_{int}}{K_{PDR1} + D_{int}} - \delta_{PDR1} PDR1$$

$$\frac{dPDR3}{dt} = \alpha_{PDR3} \frac{(PDR1 + PDR3)^{n_{PDR3}}}{K_{PDR3}^{n_{PDR3}} + (PDR1 + PDR3)^{n_{PDR3}}} - \delta_{PDR3} PDR3 \tag{3}$$

$$\frac{dPDR5}{dt} = \alpha_{PDR5} \frac{(PDR1 + PDR3)^{n_{PDR5}}}{K_{PDR5}^{n_{PDR5}} + (PDR1 + PDR3)^{n_{PDR5}}} - \delta_{PDR5} PDR5$$

$$\frac{dD_{int}}{dt} = k_{diff}(D_{ext} - D_{int}) - k_{pump} PDR5 \left(\frac{D_{int}}{K_{int} + D_{int}} \right)$$

where PDR1 and the intracellular drug concentration (D_{int}) were incorporated as variables. α_0 is the basal rate of PDR1 transcription, D_{ext} the extracellular drug concentration, k_{diff} the rate of passive diffusion of the drug across the cellular membrane, k_{int} half-maximum saturation coefficient for the PDR5 efflux pump, and k_{pump} the efflux rate of the drug via PDR5 efflux pump. It was assumed that drug entry and exit from the cells occurred through a combination of passive and active transport, and that the activation of PDR1 by the drug can be described by Michaelis–Menten kinetics. The PDR5 transcriptional network model predicted that PDR5 expression level would increase after application of the drug and that cell population fitness would oscillate before stabilizing during drug treatment.

To confirm the predictions from the PDR5 transcriptional network model, a synthetic gene circuit (**Figure 2b**) was constructed with molecular cloning techniques [38] and integrated into a yeast strain without a native PDR5 gene [27]. The construction technique ran multiple overlap PCR steps, where two fragments with overlapping regions on their ends were amplified together initially without primers, using the overlapping sequence as a *de facto* primer. Homologous recombination facilitated the integration of the synthetic gene circuit into the yeast genome. This synthetic PDR gene circuit has rtTA activating its own expression through a tetracycline-inducible promoter (**Figure 2b**), mimicking the positive feedback

activation of PDR3 (**Figure 2a**). The Doxycycline inducible promoter regulating rtTA expression is also controlled by a *β*-estradiol inducible GEV regulator, representing PDR1, which also activates the PDR5 gene in this synthetic gene circuit [27], completing the core PDR network architecture (**Figure 2c**) [34].

The PDR synthetic gene circuit was experimentally compared to simplified PDR circuit components, like direct activation (DA: PDR1 directly activates PDR5), cascade (CAS: PDR1 activates PDR5 through PDR3), a cascade with positive feedback loop (CAS + PF: PDR1 activates PDR5 through PDR3, with PDR3 activating its own expression), and a feedforward loop (FFL: PDR1 activates PDR5 directly as well as indirectly through PDR3) [27]. The FFL + PF circuit represented the PDR network. This separation of components tested the effect of specific network motifs in drug resistance and gene expression compared to the full PDR synthetic circuit. Direct activation in the FFL circuit was found experimentally to increase the speed of expression changes compared to indirect activation of PDR5. Direct activation was required for PDR5-mediated drug resistance. Indirect activation enhanced drug resistance, which provided evidence that the delayed reduction in PDR5 expression protected cells from the toxic effects of drug (Cycloheximide) exposure (**Figure 3a**). **Figure 3b** shows that strains carrying a gene circuit with a positive feedback loop grew faster in the presence of drug compared to strains in which this circuit architecture was missing. This provided evidence that the positive feedback regulation of PDR5 expression protects cells from drug exposure. Finally, reducing the strength of positive feedback regulation through mutation decreased drug resistance (**Figure 3c**).

Overall, the PDR network was recapitulated in mathematical and synthetic gene circuit models that demonstrated that the network architecture is optimized for drug resistance, with gene expression noise making important contributions to fitness during drug treatment.

2.3 Experiments and computational models of mammalian negative and positive feedback gene circuits

The yeast NF gene circuit was transferred to mammalian cells to test an organism transfer workflow using design and optimization cycles aided by computational modeling [40]. This workflow led to updates to the yeast NF gene circuit, including the optimization of the tet promoter operator site locations, the introduction of an intron upstream of the regulator, codon optimization for mammalian translation,
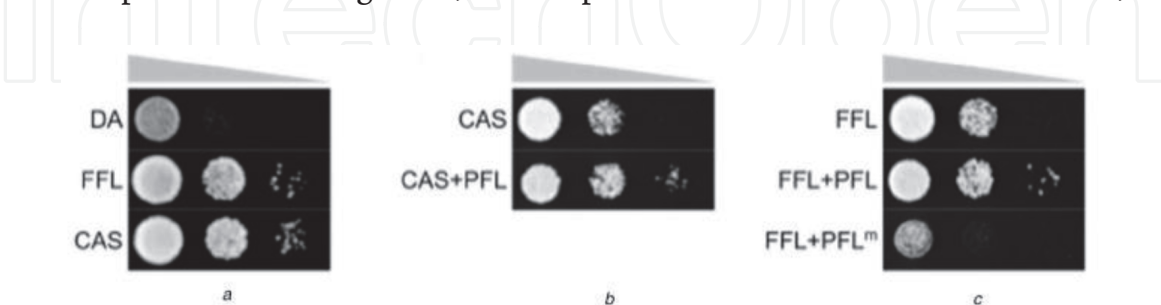

Figure 3.
*(a) Genetically engineered yeast strains with direct activation (DA) and indirect activation networks (FFL and CAS). (b) Yeast strains with indirect activation alone (CAS) or combined with positive feedback (CAS + PFL). (c) Yeast strains with feedforward activation alone (FFL) or combined with positive feedback (FFL +PFL) or mutated positive feedback (FFL+PFLm). Plates contain 5 μg/ml doxycycline (inducer), 0.025 (a) or 0.05 μg/ml (b, c) Cycloheximide (drug) and no β-estradiol (inducer). Gray triangles represent decreasing density of the spotted cell culture, 1:10 serial dilutions from OD600 0.1 to 1 x 10–3. From **Figure 6** "indirect activation and positive feedback enhance drug resistance in spot assay experiment" by Brendan Camellato et al. in [27] located at https://ietresearch.onlinelibrary.wiley.com/doi/10.1049/enb.2019.0009 under a CC BY-NC-ND 3.0 license.*

and the addition of a Kozak sequence near the start codon, which was stably but randomly transfected into MCF-7 breast cancer cells. This NF circuit in mammalian cells exhibited a linear dose response with low gene expression noise, similar to the NF circuit in yeast cells [29]. Though the adaptability of the yeast NF circuit to mammalian cells did not require any additional design features, optimization of parts responsible for gene expression and protein location was required to replicate the features of the yeast NF circuit. These results support the "abstraction principle" in the field of synthetic biology, namely that different parts of a biological network can be optimized for improved functionality in new settings, while leaving the original network design intact [40].

In a subsequent study, the yeast PF circuit was transferred to mammalian cells, which was coupled with a Flp-recombinase site-specific integration system (**Figure 4a**) [39]. This mammalian positive feedback (mPF) circuit displayed a sigmoidal mean gene expression dose response (**Figure 4b**). Gene expression noise increased at intermediate inducer (Doxycycline) levels (**Figure 4c**), with broad unimodal gene expression distributions (**Figure 4d**). A lack of bimodality is unexpected for an induced bistable circuit and may have been attributed to similar cellular growth and cellular memory time scales.

The previously reported mammalian negative feedback circuit was also connected to the Flp-In integration system in Chinese Hamster Ovary (CHO) cells (**Figure 5a**) [40]. This negative feedback circuit was separately integrated into the same genomic site as the mPF circuit and subsequently called the mammalian negative feedback (mNF) circuit. The mNF circuit displayed a linear dose response in mean gene expression (**Figure 5b**). Gene expression noise was low across all inducer (Doxycycline) levels (**Figure 5c**) with narrow gene expression distributions (**Figure 5d**).
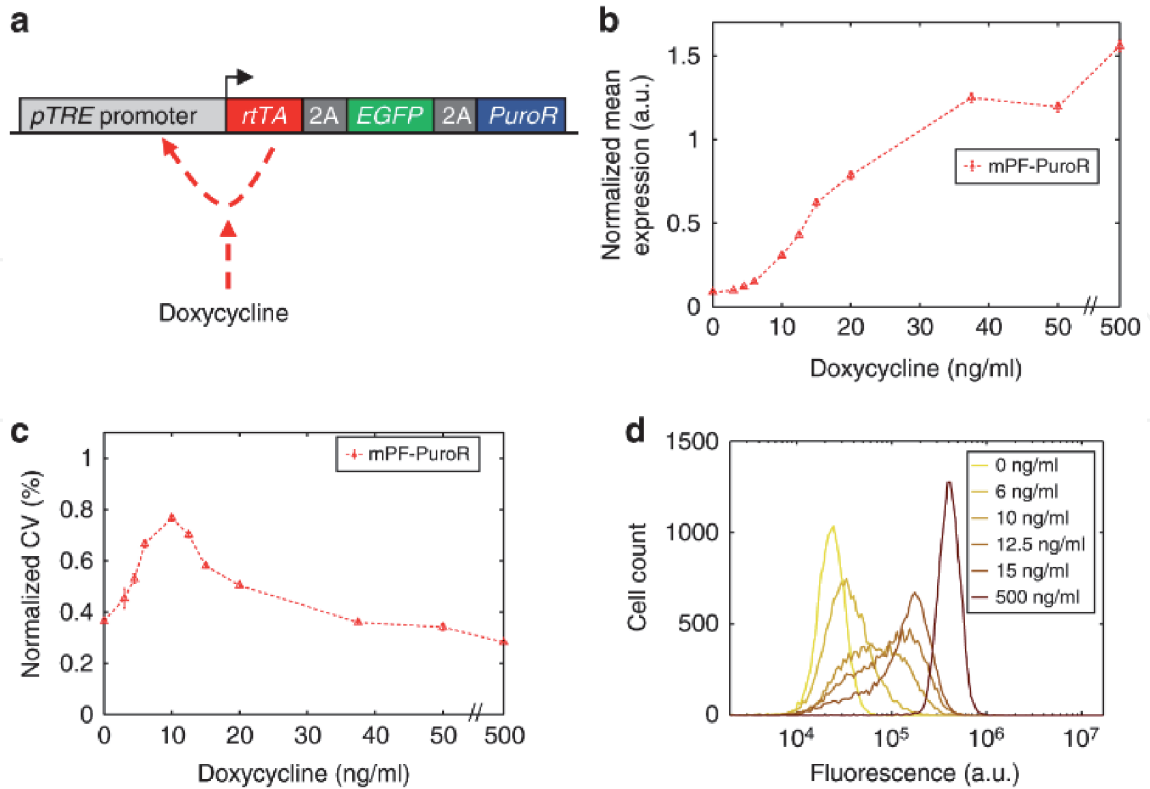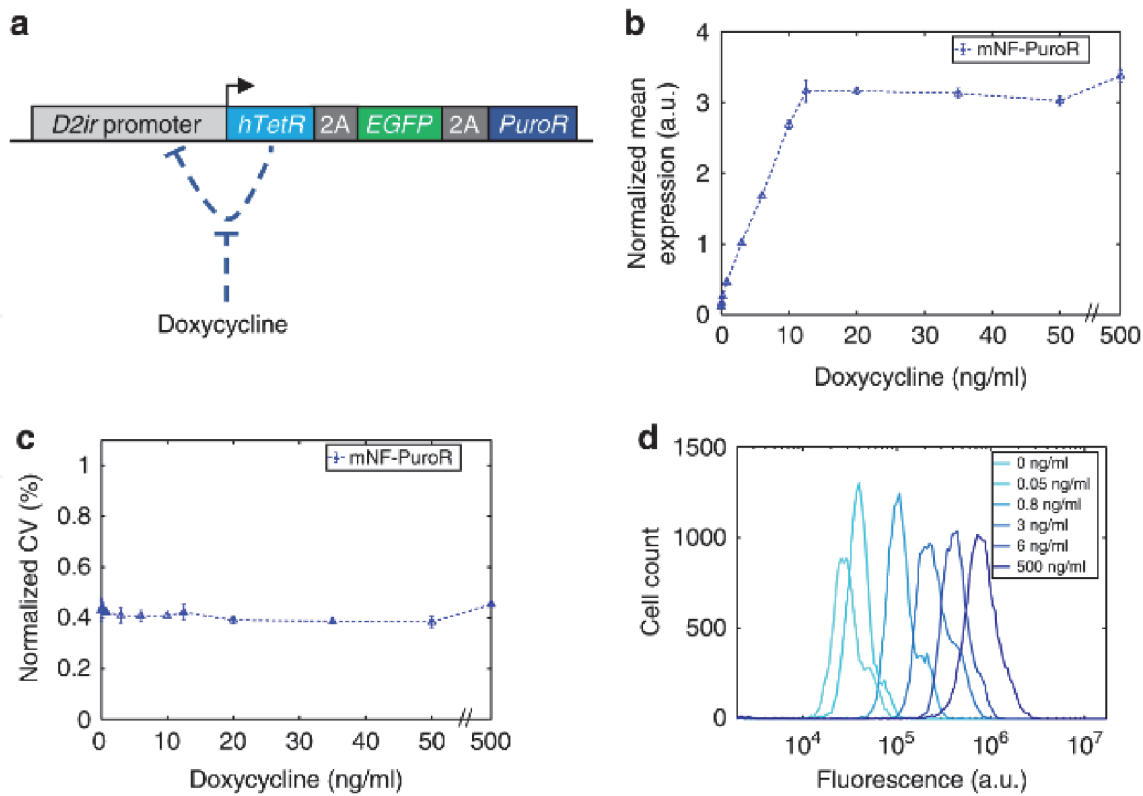

Figure 4.
*(a) Schematic of the mammalian positive feedback (mPF) synthetic gene network. (b-d) dose responses. (b) Mean gene expression. (c) Gene expression noise determined from the coefficient of variation (CV). (d) Single-cell gene expression distributions. From **Figure 2** "dose–response of the mPF-PuroR gene circuit" by Farquhar, K.S. et al. in [39] located at https://www.nature.com/articles/s41467-019-10330-w under a CC BY 4.0 license.*

Figure 5.
(a) Schematic of the mammalian negative feedback (mNF) synthetic gene network. (b-d) dose responses. (b) Mean gene expression. (c) Gene expression noise determined from the coefficient of variation (CV). (d) Single-cell gene expression distributions. From **Figure 3** *"dose–response of the mNF-PuroR gene circuit" by Farquhar, K.S. et al. in [39] located at https://www.nature.com/articles/s41467-019-10330-w under a CC BY 4.0 license.*

The mPF and mNF gene circuits controlled the EGFP fluorescent protein and the PuroR drug resistance gene each separated by self-cleaving 2A motifs [39, 40]. The, the integration into the same genomic site and the introduction of self-cleaving 2A motifs and the PuroR drug resistance gene did not affect the function of these circuits.

In an evolution experiment with multiple drug (Puromycin) concentrations, the mNF and mPF circuits were tuned to the same mean expression level to decouple gene expression noise from the mean gene expression prior to drug treatment [39]. After adaptation, the drug was removed while induction was either maintained or removed. Finally, the adapted populations were retreated with the previous level of drug to uncover potential adaptation mechanisms. The evolution experiment demonstrated that low gene expression noise from the mNF circuit was beneficial in adaptation compared to mPF under low levels of drug [39]. In contrast, the high noise from the mPF circuit was beneficial compared to the mNF circuit under high levels of drug.

Mutations were found in the TetR regulator gene from the mNF circuit that knocked out repression, which explained why the mNF populations maintained high expression with or without inducer after the temporary removal of the drug. The mPF circuits did not mutate, which was consistent with the drug retreatment period where uninduced mPF populations struggled to adapt while the induced populations adapted faster [39]. Overall, the decoupling of gene expression noise from the mean demonstrated the power of using synthetic gene circuits to uncover novel insights into mammalian drug resistance.

A stochastic population dynamics model was developed to predict the emergence and switching dynamics of persister (P), nongenetically drug-resistant (N),

and genetically drug-resistant (G) subpopulations (**Figure 6a**), which was described mathematically by the following set of coupled ODEs [39]:

$$\frac{dP}{dt} = r_{P,N}N - r_{N,P}P - r_{G,P}P$$

$$\frac{dN}{dt} = -r_{P,N}N + r_{N,P}P - r_{G,N}N + k_N N - g_N N \qquad (4)$$

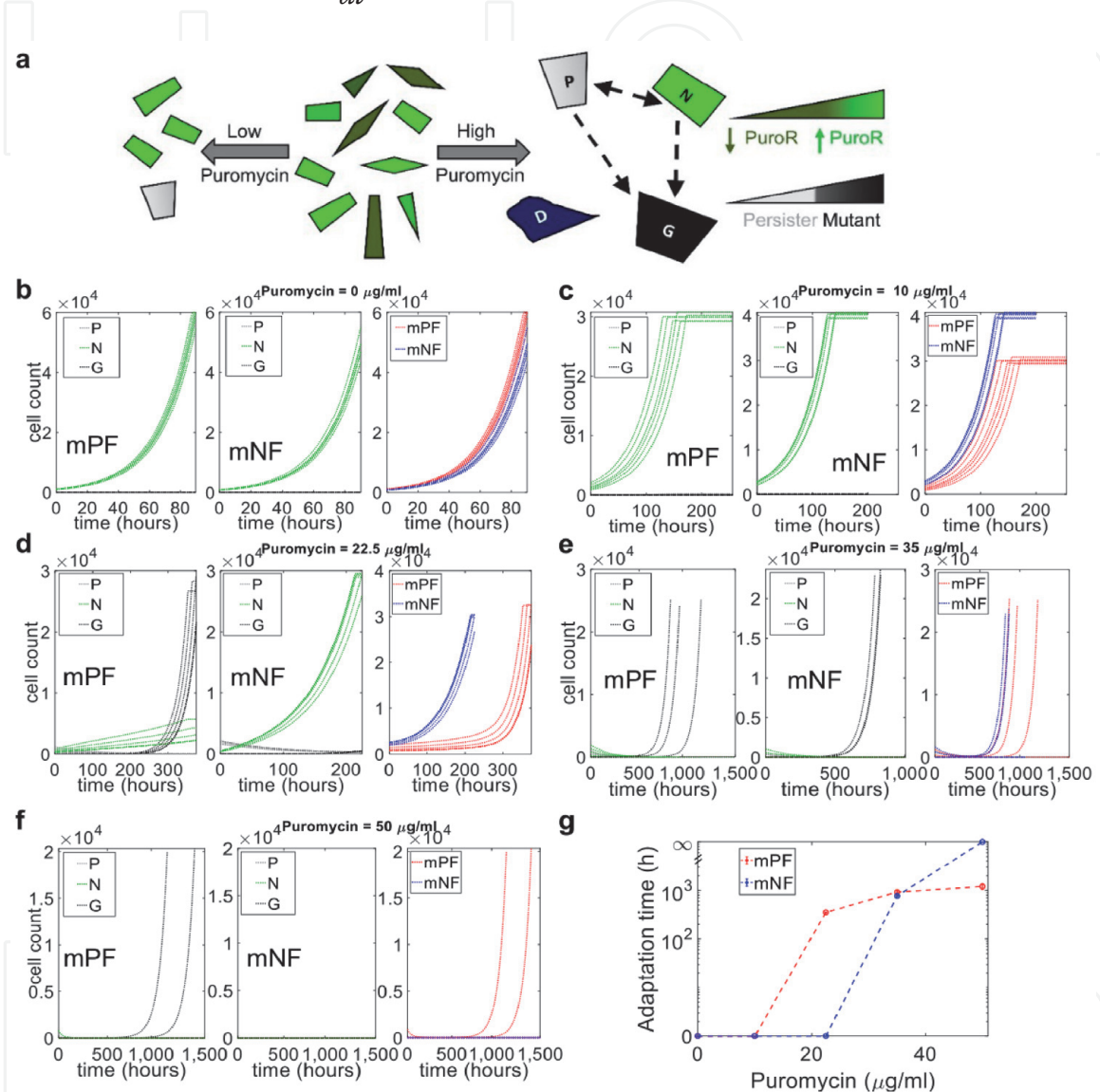$$\frac{dG}{dt} = r_{G,P}P + r_{G,N}N + k_G G - g_G G$$

Figure 6.
*(a) Schematic depicting the effects of drug (Puromycin) concentration on Chinese hamster ovary (CHO) cell population composition and survival. Nongenetically drug-resistant cells (green cells – Brighter cells have higher PuroR expression level and are therefore more resistant) and nongrowing persister cells (gray cells) can switch phenotypes (dashed bidirectional arrow). Persister cells and growing nongenetically resistant cells can also become stably drug-resistant cells (black cells). When no drug is present, a genetically identical (clonal) cell population with heterogeneous gene expression exists (center). Under low drug treatment conditions (left arrow), cells with low PuroR expression perish and a small fraction of the surviving clonal cells become persister cells. For high drug treatment conditions (right arrow), only cells with high PuroR expression levels can survive drug treatment while the rest die (dark blue cells), and a higher fraction of the surviving cells become persisters. As persister and nongenetically resistant cells can become stably drug resistant, the population on the right panel becomes increasingly heterogeneous over the course of treatment. (b-f) Representative growth curves for simulated mPF-PuroR and mNF-PuroR CHO cell populations under (b) 0, (c) 10, (d) 22.5, (e) 35, and (f) 50 μg/mL of Puromycin. Growth curves shown in panels in (b-f) correspond to: (left) mPF subpopulations, (center) mNF subpopulations, and (right) mPF and mNF populations. (g) Adaptation times corresponding to the mPF-PuroR and mNF-PuroR populations shown in panels (b-f). From **Figure 6** "Modeling the adaptation of mPF-PuroR and mNF-PuroR cells in various concentrations of Puromycin" by Farquhar, K.S. et al. in [39] located at https://www.nature.com/articles/s41467-019-10330-w under a CC BY 4.0 license.*

where $r_{i,j}$ is transition rate from genotype or phenotype j to i, k_i is the growth rate of i, and g_i is the death rate of i. Noise was incorporated into this model by drawing the parameters describing the initial number of cells that survived Puromycin treatment and the carrying capacity of the cell culture environment from a normal distribution. Numerical simulations of Eq. (4) are shown in **Figure 6b–f**. These simulation results agreed with the data from the evolution experiments [39]. The modeling indicated that nongenetic phenotypic variability could facilitate the adaptation of the mPF and mNF strains to lower drug concentrations (**Figure 6c**), but that population dynamics in terms of the P to G conversion was required to capture the long experimental adaptation times at higher drug concentrations (**Figure 6d–g**).

3. Optogenetic gene circuits

A major focus of synthetic biology has been to engineer gene circuits to control cellular processes. This has mainly been achieved through small molecules that activate or inactivate various components of synthetic gene circuits [17, 19, 41–46]. Chemical stimuli has many advantages, including easy titration for inducing gene expression over large dynamic ranges, characterized affinity for existing proteins, and minimal off-target effects [9]. However, controlling gene circuits with chemicals is often not instantaneous and makes it difficult to control individual cells in a population.

Light stimulus can achieve many of the same advantages as chemicals without the above limitations. Like the cellular proteins that respond to chemical stimuli, proteins have been found in nature that respond to light [47, 48]. The discovery of light-activated proteins provided the elements necessary to build optogenetic gene circuits. By engineering light-responsive elements with existing components in gene circuits, synthetic biologists were able to adapt endogenous proteins from natural organisms to experimental model organisms, including yeast [7] and mammalian cell lines [49].

The use of light-inducible systems in eukaryotic organisms has expanded to cover nearly as many applications as chemical systems, including the control of gene expression, protein alterations, metabolic reactions, epigenetic states, and animal behavior [50–55]. A common theme among light-activated and chemical-induced circuits is the genetic architecture of the system. For chemically regulated gene circuits, classic engineering architectures [26] have been produced including negative regulation, positive regulation, positive feedback, negative feedback, and many others [39, 40, 42, 56–59]. Optogenetic systems have begun to incorporate these circuit architectures [49, 60]. Optogenetic tools respond to a variety of wavelengths of light [61, 62] and can be used transiently [63, 64] or as stable systems [60].

Negative feedback is an important gene circuit architecture that has been implemented in optogenetic circuits. Negative feedback is a desirable architecture in synthetic biology because it provides two advantages: 1) negative feedback reduces gene expression noise and 2) negative feedback allows tunability of system output to a "transfer function", which describes the relationship between an input and an output function [65, 66]. In synthetic biology, many systems are designed with desired inputs and outputs in mind and therefore knowing the relationship or transfer function between these variables is crucial [67]. Additionally, such features also occur in natural systems [68, 69], which synthetic systems are often designed to mimic [14]. The negative feedback circuit architecture has been engineered into synthetic gene circuits in bacterial, fungal, and mammalian systems, all controllable

by small chemical molecules [29, 40, 70, 71]. This circuit architecture was recently engineered in an optogenetic system and found to offer many of the same advantages as the chemical-induced negative feedback gene circuits; namely, low gene circuit noise, wide system tunability, and a characterized transfer function between input and output (i.e., light and a fluorescence reporter) [60].

The optogenetic NF system (**Figure 7A**) was inspired from previous chemical gene circuits [40] and from computational modeling [57]. A well-known tetracycline-responsive system [72, 73] provided the foundation to engineer a light-responsive system, by fusing the TetR protein with a LOV2 domain [52, 74, 75] and either a degradation tag [74] or a small peptide [76] that inhibits TetR (**Figure 7B**). When light is absent, the degradation tag or the inhibitory peptide remains hidden. When blue light is present, the LOV2 protein undergoes a confirmational change and reveals one of the two domains. By employing this engineered light protein, an optogenetic gene circuit can be constructed with operator sites upstream of the gene for this protein to allow down regulation of its own expression (as well as another functional gene). The light stimulus can then be used to control gene expression output with the benefits of low noise and titratable expression levels.

Computational modeling was used to investigate how system performance could be enhanced in the optogenetic NF system [60]. This methodology of build, model, improve is crucial when developing synthetic gene circuits. To achieve this, the design and construction of the optogenetic NF system focused on changes that could decrease gene circuit noise, lower basal expression of the circuits, increase fold-change of the circuit, and enhance the range of circuit response to stimuli. A
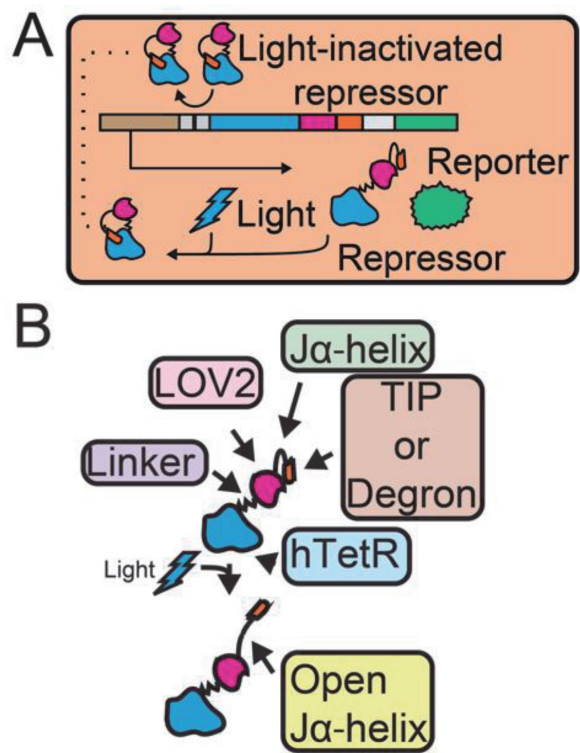

Figure 7.
(A) Schematic illustrating an optogenetic gene circuit with a negative feedback architecture. Specifically, this architecture produces a transcription factor (blue) that inhibits its own production. This transcription factor is also engineered to have a light-responsive domain (pink) and inhibitory peptide (orange). When light is added to the system, a confirmational change occurs and the hidden inhibitory peptide is exposed to inhibit the DNA-binding function of the transcription factor. When this occurs, increased transcription and translation occurs for the reporter gene. (B) Schematic illustration of the transcription factor shown in (A). The transcription factor is the TetR protein fused with a linker peptide which is fused with the light-responsive LOV2 domain, which contains a Jα-helix that is fused with a functional domain such as a degradation tag or an inhibitory peptide (TIP). When light is added, the Jα-helix opens exposing the functional domain. Figure used with permission from Guinn [55].
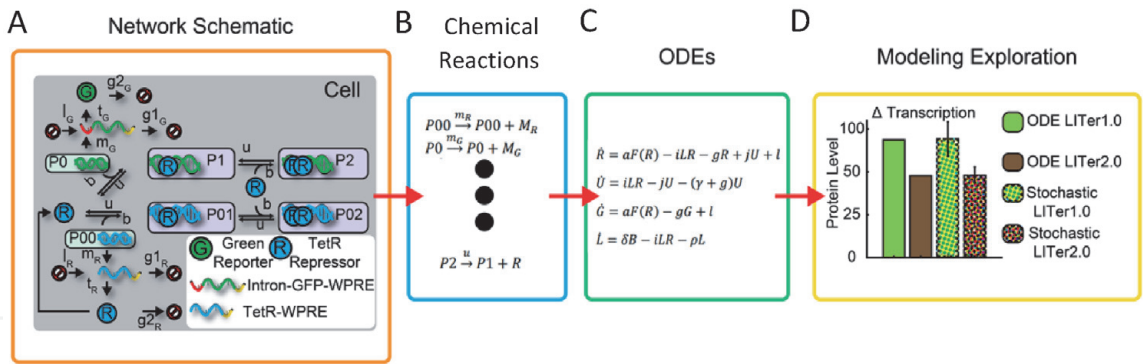
Figure 8.
The "pipeline" used to model the NF optogenetic gene circuit. The network schematic of the system of interest includes transcription factors, promoters that can be bound by transcription factors, reporters, RNA, etc. (A). The network schematic is converted into a set of chemical reactions (B). These chemical reactions are then converted into a set of ordinary differential equations (ODEs) (C). Finally, the ODEs are simulated numerically, providing quantitative predictions that can be used to improve the optogenetic gene circuit (D). Figure used with permission from Guinn [55].

quantitative gene expression model identified parameters that optimized the performance of the optogenetic NF system. The "pipeline" for modeling the NF optogenetic gene circuit is shown in **Figure 8**. The NF optogenetic circuit was represented by a network schematic of the main optogenetic gene circuit's components (**Figure 8A**). These components were formalized as a set of chemical reactions (**Figure 8B**). The chemical reactions were then described by a system of ordinary differential equations (**Figure 8C**), which was solved numerically [57]. Lastly, the equations describing each reaction were explored to investigate whether a given optogenetic gene circuit component should be changed experimentally (**Figure 8D**). The design of the optogenetic NF system was improved by changing the transcription and translation rates of the optogenetic inhibitory protein.

In addition to validation and improving optogenetic gene circuits, various architectures can be utilized for expressing functional proteins at precise levels inside of single cells. Controlling gene expression in single cells can allow for exploration of phenotypic landscapes as a function of protein levels and time. For example, the optogenetic NF system was used to control the mutated oncogene KRAS (G12V), which showed expression and function could be controlled in a dose-responsive manner with low optogenetic gene circuit noise [60]. This system can be modified to contain any functional gene allowing single-cell gene expression studies using microscopy equipment such as digital mirror device (DMD) [77]. DMD technology can allow system feedback for controlling optogenetic gene circuits *in silico* at the computer-microscopy interface [78] and *in vitro* using gene architecture designs responsive to light. Coupling technology like the DMD with optogenetic gene circuits like the optogenetic NF system will allow researchers to better understand cellular processes and single-cell biology.

Overall, optogenetic gene circuits allow researchers to perturb single cells to distinguish between individual and population-level behavior. Optogenetic tools are anticipated to be important for elucidating mechanisms in drug resistance and cancer metastasis, where single-cell behavior and spatial–temporal factors may dictate biological fate.

4. Conclusions

The ability of synthetic gene circuits to fulfill engineered design principles and facilitate scientific discoveries is expected to grow over time. However, evolutionary forces can undermine the integrity of synthetic gene circuits [25, 32]. It will be

crucial to design gene circuits in the future to mitigate the effects of evolution to maintain their functional integrity. One approach is to use DNA sponges to change the response of gene circuits while lowering protein toxicity [79]. Another approach is to use evolution itself to repair broken synthetic gene circuit components, resulting in more robust gene circuits [31]. Overall, as the library of biological parts increases, the discovery of new "BioBricks" (standardized and interchangeable gene circuits components) will aid in resolving the integrity issues presently associated with synthetic gene circuits. Genomic mining is a promising approach for discovering BioBricks, including identifying novel TetR-family regulators from prokaryotic genomes [80] and CRISPR-Cas systems in microbes [81].

Clinical applications of synthetic gene circuits will continue to expand and could lead to successful treatments for various diseases, including autoimmune disorders [82] and cancers [83]. CAR-T technologies to fight cancer increasingly include synthetic gene circuits and synthetic intercellular pathways to avoid adverse inflammatory reactions that damage healthy cells and to improve the targeting of cancer cells [83, 84]. Additionally, investigating drug resistance in microbial pathogens will require gene circuits that can be introduced into pathogens, which have native gene networks relevant to drug resistance that are complex and incompletely characterized. Relatedly, increasing complexity in gene circuits remains a challenge and will require multiple orthogonal components [80, 85] as well as more advanced computational methods to predict the dynamics of large-scale, nonlinear networks [86]. Ultimately, improvements in our ability to model, design, and construct synthetic gene circuits will benefit biomedical applications as well as increase our understanding of natural gene networks.

Optogenetic gene circuits allow researchers to utilize the strengths that have been developed through two decades of synthetic biology research, as well as to achieve more precise control of living cells. The use of light as a stimulus enables the single-cell control of gene circuit response, which can complement existing systems to study cell populations. The generation of single-cell data will allow researchers to address questions on how individual cells give rise to population level phenomenon and how neighboring cells affect adjacent or distal cells. Answering such questions will be important for extracting information on biological processes such as tissue development [87], epithelial-to-mesenchymal transition [88], and the effects of the microenvironment on cancer progression [89, 90].

While using optogenetic tools will be important for answering a broad range biological questions and for biomedical applications, challenges remain in terms of the scalability and precision of cellular control. There have been applications of optogenetic technology that address these challenges individually. For instance, the light plate apparatus (LPA) [91] is a simple to construct and inexpensive system that can be adapted and used for scaling light-induced conditions *in vitro*. Additionally technology like digital micromirror devices (DMDs) [92] have been used to control single cells in real time. The LPA technology offers scalability but is currently limited in the precision of single-cell control. The DMD on the other hand offers precise single-cell control but is limited by scalability of conditions that can be controlled in a single experiment. Coupling these two types of tools, or their subsequent technological successors, may allow researchers to maximize scalability and optogenetic gene circuit control.

Acknowledgements

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References

[1] Sorg RA, Gallay C, Van Maele L, Sirard JC, Veening JW. Synthetic gene-regulatory networks in the opportunistic human pathogen Streptococcus pneumoniae. Proc Natl Acad Sci U S A. 2020;117(44): 27608-27619.

[2] Angelici B, Mailand E, Haefliger B, Benenson Y. Synthetic biology platform for sensing and integrating endogenous transcriptional inputs in mammalian cells. Cell Rep. 2016;16(9):2525-2537.

[3] Siu Y, Fenno J, Lindle JM, Dunlop MJ. Design and selection of a synthetic feedback loop for optimizing biofuel tolerance. ACS Synth Biol. 2018; 7(1):16-23.

[4] Honjo H, Iwasaki K, Soma Y, Tsuruno K, Hamada H, Hanai T. Synthetic microbial consortium with specific roles designated by genetic circuits for cooperative chemical production. Metab Eng. 2019;55: 268-275.

[5] Daniel R, Rubens JR, Sarpeshkar R, Lu TK. Synthetic analog computation in living cells. Nature. 2013;497(7451): 619-623.

[6] Huang H, Liu Y, Liao W, Cao Y, Liu Q, Guo Y, et al. Oncolytic adenovirus programmed by synthetic gene circuit for cancer immunotherapy. Nat Commun. 2019;10(1):4801.

[7] Shimizu-Sato S, Huq E, Tepperman JM, Quail PH. A light-switchable gene promoter system. Nat Biotechnol. 2002;20(10):1041-1044.

[8] Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A. 1992;89(12): 5547-5551.

[9] Hasty J, McMillen D, Collins JJ. Engineered gene circuits. Nature. 2002; 420(6912):224-230.

[10] Kaern M, Elston TC, Blake WJ, Collins JJ. Stochasticity in gene expression: From theories to phenotypes. Nature Reviews Genetics. 2005;6(6):451-464.

[11] Fraser D, Kaern M. A chance at survival: Gene expression noise and phenotypic diversification strategies. Molecular Microbiology. 2009;71(6): 1333-1340.

[12] Farquhar KS, Koohi SR, Charlebois DA. Does transcriptional heterogeneity facilitate the development of genetic drug resistance? BioEssays. 2021:e2100043.

[13] Brock A, Chang H, Huang S. Non-genetic heterogeneity – A mutation-independent driving force for the somatic evolution of tumours. Nature Reviews Genetics. 2009;10(5):336-342.

[14] Farquhar KS, Flohr H, Charlebois DA. Advancing antimicrobial resistance research through quantitative Modeling and synthetic biology. Frontiers in Bioengineering and Biotechnology. 2020;8:583415.

[15] McAdams HH, Shapiro L. Circuit simulation of genetic networks. Science. 1995;269(5224):650-656.

[16] Becskei A, Serrano L. Engineering stability in gene networks by autoregulation. Nature. 2000;405 (6786):590-593.

[17] Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in Escherichia coli. Nature. 2000;403 (6767):339-342.

[18] Weisberg RA. A genetic switch: Phage lambda and higher organisms. Mark Ptashne. The Quarterly Review of Biology. 1994;69(2):267-268.

[19] Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional

regulators. Nature. 2000;403(6767): 335-338.

[20] Weber W, Schoenmakers R, Keller B, Gitzinger M, Grau T, Daoud-El Baba M, et al. A synthetic mammalian gene circuit reveals antituberculosis compounds. Proc Natl Acad Sci U S A. 2008;105(29):9994-9998.

[21] Jancik S, Drabek J, Radzioch D, Hajduch M. Clinical relevance of KRAS in human cancers. J Biomed Biotechnol. 2010;2010:150960.

[22] Chen S, Weitemier AZ, Zeng X, He L, Wang X, Tao Y, et al. Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics. Science. 2018;359(6376): 679-684.

[23] Eickelbeck D, Rudack T, Tennigkeit SA, Surdin T, Karapinar R, Schwitalla JC, et al. Lamprey Parapinopsin ("UVLamP"): A Bistable UV-sensitive Optogenetic switch for ultrafast control of GPCR pathways. ChemBioChem. 2020;21(5): 612–617.

[24] Becskei A, Séraphin B, Serrano L. Positive feedback in eukaryotic gene networks: Cell differentiation by graded to binary response conversion. EMBO J. 2001;20(10):2528–2535.

[25] Nevozhay D, Adams RM, Van Itallie E, Bennett MR, Balazsi G. Mapping the environmental fitness landscape of a synthetic gene circuit. PLoS Computational Biology. 2012;8(4): e1002480.

[26] Alon U. Network motifs: Theory and experimental approaches. Nat Rev Genet. 2007;8(6):450-461.

[27] Camellato B, Roney IJ, Azizi A, Charlebois D, Kaern M. Engineered gene networks enable non-genetic drug resistance and enhanced cellular robustness. Engineering Biology. 2019;3 (4):72-79.

[28] Nevozhay D, Adams RM, Van Itallie E, Bennett MR, Balazsi G. Mapping the environmental fitness landscape of a synthetic gene circuit. PLoS Comput Biol. 2012;8(4):e1002480.

[29] Nevozhay D, Adams RM, Murphy KF, Josic K, Balázsi G. Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression. Proc Natl Acad Sci USA. 2009;106:5123-5128.

[30] Baron U, Gossen M, Bujard H. Tetracycline-controlled transcription in eukaryotes: Novel transactivators with graded transactivation potential. Nucleic Acids Res. 1997;25(14): 2723-2729.

[31] Gouda MK, Manhart M, Balázsi G. Evolutionary regain of lost gene circuit function. Proc Natl Acad Sci USA. 2019; 116(50):25162-25171.

[32] Gonzalez C, Ray JC, Manhart M, Adams RM, Nevozhay D, Morozov AV, et al. Stress-response balance drives the evolution of a network module and its host genome. Mol Syst Biol. 2015;11(8): 827.

[33] Charlebois DA, Balazsi G, Kaern M. Coherent feedforward transcriptional regulatory motifs enhance drug resistance. Physical Review E. 2014;89 (5):052708.

[34] Balzi E, Goffeau A. Yeast multidrug resistance: The PDR network. J Bioenerg Biomembr. 1995;27(1):71-76.

[35] Misra S, Ghatak S, Toole B. Regulation of MDR1 expression and drug resistance by a positive feedback loop involving hyaluronan, phosphoinositide 3-kinase, and ErbB2. J Biol Chem. 2005;280:20310-20315.

[36] Gillespie DT. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. J Comput Phys. 1976;22: 403-434.

[37] Gillespie DT. Exact stochastic simulation of coupled chemical reactions. J Phys Chem. 1977;81: 2340-2361.

[38] Sambrook J. Molecular cloning: A laboratory manual: Third edition. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press [2001] ©2001; 2001.

[39] Farquhar KS, Charlebois DA, Szenk M, Cohen J, Nevozhay D, Balazsi G. Role of network-mediated stochasticity in mammalian drug resistance. Nat Commun. 2019;10(1):2766.

[40] Nevozhay D, Zal T, Balazsi G. Transferring a synthetic gene circuit from yeast to mammalian cells. Nat Commun. 2013;4:1451.

[41] Guinn M, Bleris L. Biological 2-input decoder circuit in human cells. ACS Synth Biol. 2014;3(8):627-633.

[42] Bleris L, Xie Z, Glass D, Adadey A, Sontag E, Benenson Y. Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. Mol Syst Biol. 2011;7:519.

[43] Auslander S, Stucheli P, Rehm C, Auslander D, Hartig JS, Fussenegger M. A general design strategy for protein-responsive riboswitches in mammalian cells. Nat Methods. 2014;11(11): 1154-1160.

[44] Thibodeaux GN, Cowmeadow R, Umeda A, Zhang Z. A tetracycline repressor-based mammalian two-hybrid system to detect protein-protein interactions in vivo. Anal Biochem. 2009;386(1):129-131.

[45] Kramer BP, Fussenegger M. Hysteresis in a synthetic mammalian gene network. Proc Natl Acad Sci U S A. 2005;102(27):9517-9522.

[46] Bacchus W, Lang M, El-Baba MD, Weber W, Stelling J, Fussenegger M. Synthetic two-way communication between mammalian cells. Nat Biotechnol. 2012;30(10):991-996.

[47] Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci. 2005;8(9):1263-1268.

[48] Zoltowski BD, Crane BR. Light activation of the LOV protein vivid generates a rapidly exchanging dimer. Biochemistry. 2008;47(27):7012-7019.

[49] Wang X, Chen X, Yang Y. Spatiotemporal control of gene expression by a light-switchable transgene system. Nat Methods. 2012;9 (3):266-269.

[50] Muller K, Engesser R, Metzger S, Schulz S, Kampf MM, Busacker M, et al. A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells. Nucleic Acids Res. 2013;41(7):e77.

[51] Levskaya A, Weiner OD, Lim WA, Voigt CA. Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature. 2009;461 (7266):997-1001.

[52] Renicke C, Schuster D, Usherenko S, Essen LO, Taxis C. A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. Chem Biol. 2013;20(4):619-626.

[53] Ye H, Daoud-El Baba M, Peng RW, Fussenegger M. a synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. Science. 2011;332(6037):1565-1568.

[54] Folcher M, Oesterle S, Zwicky K, Thekkottil T, Heymoz J, Hohmann M, et al. Mind-controlled transgene expression by a wireless-powered optogenetic designer cell implant. Nat Commun. 2014;5:5392.

[55] Guinn MT. Engineering Human Cells with Synthetic Gene Circuits

Elucidates How Protein Levels Generate Phenotypic Landscapes [Ph.D. Thesis]. Ann Arbor: State University of New York at Stony Brook; 2020.

[56] Zhao W, Bonem M, McWhite C, Silberg JJ, Segatori L. Sensitive detection of proteasomal activation using the Deg-on mammalian synthetic gene circuit. Nat Commun. 2014;5:3612.

[57] Charlebois DA, Diao J, Nevozhay D, Balazsi G. Negative regulation gene circuits for efflux pump control. Methods Mol Biol. 2018;1772:25-43.

[58] May T, Eccleston L, Herrmann S, Hauser H, Goncalves J, Wirth D. Bimodal and hysteretic expression in mammalian cells from a synthetic gene circuit. PLoS One. 2008;3(6):e2372.

[59] Li Y, Moore R, Guinn M, Bleris L. Transcription activator-like effector hybrids for conditional control and rewiring of chromosomal transgene expression. Sci Rep. 2012;2:897.

[60] Guinn MT, Balazsi G. Noise-reducing optogenetic negative-feedback gene circuits in human cells. Nucleic Acids Res. 2019;47(14):7703-14.

[61] Lee D, Hyun JH, Jung K, Hannan P, Kwon HB. A calcium- and light-gated switch to induce gene expression in activated neurons. Nat Biotechnol. 2017; 35(9):858-863.

[62] Polstein LR, Gersbach CA. A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. Nat Chem Biol. 2015;11(3):198-200.

[63] Ma Z, Du Z, Chen X, Wang X, Yang Y. Fine tuning the LightOn light-switchable transgene expression system. Biochem Biophys Res Commun. 2013; 440(3):419-423.

[64] Chen X, Wang X, Du Z, Ma Z, Yang Y. Spatiotemporal control of gene expression in mammalian cells and in

mice using the LightOn system. Curr Protoc Chem Biol. 2013;5(2):111-129.

[65] 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(4): 449-455.

[66] Bradley RW, Buck M, Wang B. Tools and principles for microbial gene circuit engineering. J Mol Biol. 2016;428 (5 Pt B):862-888.

[67] Sadat Mousavi P, Smith SJ, Chen JB, Karlikow M, Tinafar A, Robinson C, et al. A multiplexed, electrochemical interface for gene-circuit-based sensors. Nat Chem. 2020;12(1):48-55.

[68] Harris SL, Levine AJ. The p53 pathway: Positive and negative feedback loops. Oncogene. 2005;24(17): 2899-2908.

[69] Yu P, Kosco-Vilbois M, Richards M, Kohler G, Lamers MC. Negative feedback regulation of IgE synthesis by murine CD23. Nature. 1994;369(6483): 753-756.

[70] Deans TL, Cantor CR, Collins JJ. A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. Cell. 2007;130(2):363-372.

[71] Madar D, Dekel E, Bren A, Alon U. Negative auto-regulation increases the input dynamic-range of the arabinose system of Escherichia coli. BMC Syst Biol. 2011;5:111.

[72] Gossen M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H. Transcriptional activation by tetracyclines in mammalian cells. Science. 1995;268(5218):1766-1769.

[73] Forster K, Helbl V, Lederer T, Urlinger S, Wittenburg N, Hillen W. Tetracycline-inducible expression systems with reduced basal activity in

mammalian cells. Nucleic Acids Res. 1999;27(2):708-710.

[74] Muller K, Zurbriggen MD, Weber W. An optogenetic upgrade for the Tet-OFF system. Biotechnol Bioeng. 2015;112(7):1483-1487.

[75] Usherenko S, Stibbe H, Musco M, Essen LO, Kostina EA, Taxis C. Photo-sensitive degron variants for tuning protein stability by light. BMC Syst Biol. 2014;8:128.

[76] Klotzsche M, Berens C, Hillen W. A peptide triggers allostery in tet repressor by binding to a unique site. J Biol Chem. 2005;280(26):24591-24599.

[77] Sakai S, Ueno K, Ishizuka T, Yawo H. Parallel and patterned optogenetic manipulation of neurons in the brain slice using a DMD-based projector. Neurosci Res. 2013;75(1): 59-64.

[78] Rullan M, Benzinger D, Schmidt GW, Milias-Argeitis A, Khammash M. An Optogenetic platform for real-time, single-cell interrogation of stochastic transcriptional regulation. Mol Cell. 2018;70(4):745-756 e6.

[79] Wan X, Pinto F, Yu L, Wang B. Synthetic protein-binding DNA sponge as a tool to tune gene expression and mitigate protein toxicity. Nature Communications. 2020;11(1): 5961.

[80] Stanton BC, K NAA, Tamsir A, Clancy K, Peterson T, Voigt CA. Genomic mining of prokaryotic repressors for orthogonal logic gates. Nature Chemical Biology. 2014;10(2): 99-105.

[81] Burstein D, Harrington LB, Strutt SC, Probst AJ, Anantharaman K, Thomas BC, et al. New CRISPR-Cas systems from uncultivated microbes. Nature 2017;542(7640):237-241.

[82] Smole A, Lainscek D, Bezeljak U, Horvat S, Jerala R. A synthetic mammalian therapeutic gene circuit for sensing and suppressing inflammation. Mol Ther. 2017;25(1):102-119.

[83] Nissim L, Wu MR, Pery E, Binder-Nissim A, Suzuki HI, Stupp D, et al. Synthetic RNA-Based Immunomodulatory Gene Circuits for Cancer Immunotherapy. Cell. 2017;171 (5):1138-50 e15.

[84] Choe JH, Watchmaker PB, Simic MS, Gilbert RD, Li AW, Krasnow NA, et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. Sci Transl Med. 2021;13(591).

[85] Szenk M, Yim T, Balázsi G. Multiplexed gene expression tuning with orthogonal synthetic gene circuits. ACS Synth Biol. 2020;9(4):930-939.

[86] Bashor CJ, Patel N, Choubey S, Beyzavi A, Kondev J, Collins JJ, et al. Complex signal processing in synthetic gene circuits using cooperative regulatory assemblies. Science. 2019;364(6440):593.

[87] Mayr U, Serra D, Liberali P. Exploring single cells in space and time during tissue development, homeostasis and regeneration. Development. 2019; 146(12).

[88] Li C, Balazsi G. a landscape view on the interplay between EMT and cancer metastasis. NPJ Syst Biol Appl. 2018;4:34.

[89] Horsman MR, Vaupel P. Pathophysiological basis for the formation of the tumor microenvironment. Front Oncol. 2016;6:66.

[90] Bailey PC, Lee RM, Vitolo MI, Pratt SJP, Ory E, Chakrabarti K, et al. Single-Cell Tracking of Breast Cancer Cells Enables Prediction of Sphere Formation from Early Cell Divisions. iScience. 2018;8:29-39.

[91] Gerhardt KP, Olson EJ, Castillo-Hair SM, Hartsough LA, Landry BP, Ekness F, et al. An open-hardware platform for optogenetics and photobiology. Sci Rep. 2016;6:35363.

[92] Milias-Argeitis A, Summers S, Stewart-Ornstein J, Zuleta I, Pincus D, El-Samad H, et al. In silico feedback for in vivo regulation of a gene expression circuit. Nat Biotechnol. 2011;29(12): 1114-1116.