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Synthetic Biology: An Emerging Engineering Discipline

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Abstract

Over the past decade, synthetic biology has emerged as an engineering discipline for biological systems. Compared with other substrates, biology poses a unique set of engineering challenges resulting from an incomplete understanding of natural biological systems and tools for manipulating them. To address these challenges, synthetic biology is advancing from developing proof-of-concept designs to focusing on core platforms for rational and high-throughput biological engineering. These platforms span the entire biological design cycle, including DNA construction, parts libraries, computational design tools, and interfaces for manipulating and probing synthetic circuits. The development of these enabling technologies requires an engineering mindset to be applied to biology, with an emphasis on generalizable techniques in addition to application-specific designs. This review aims to discuss the progress and challenges in synthetic biology and to illustrate areas where synthetic biology may impact biomedical engineering and human health.

Contents

INTRODUCTION

A challenge for reviewing the field of synthetic biology lies in defining what it encompasses. Indeed, one of the most exciting aspects of synthetic biology is the diversity of applications it may enable. This opportunity is reflected in the broad backgrounds of its practitioners, which include well-established engineering disciplines such as chemical engineering, electrical engineering, computer science, and mechanical engineering, as well as branches of the biological sciences, including biophysics, biochemistry, medicine, biomedical engineering, and molecular cell biology. This diversity has led to a wide range of approaches, perspectives, and goals in the field. Nonetheless, independent of the ultimate application area, synthetic biology is characterized by a focus on constructive approaches to understanding and manipulating biological systems. This is a challenging goal, as biology is complex and the principles that determine its operation are not well elucidated.

As a result, the biological design cycle is currently slow, expensive, and laborious. Most studies are carried out empirically with a relatively small number of repurposed parts without predictive modeling. This ad hoc synthetic biology has achieved many exciting proof-of-concept circuits but will be challenging to scale to larger and more complex systems. To break through these barriers, significant efforts to develop an integrated and extensible biological design cycle are necessary (**Figure 1**). This design cycle would enable practitioners to develop high-level conceptual designs, translate these designs into potential circuit implementations using libraries of well-characterized devices, model and verify their behavior in silico, construct the designs in an automated or high-throughput fashion, and modulate and probe the resulting constructs for proper operation. Continuous feedback between multiple stages in the design cycle will be necessary to enhance their performance and integration. Furthermore, evolutionary strategies for enhancing system designs are a unique feature of biology as compared with other engineering substrates, but they are currently underutilized.

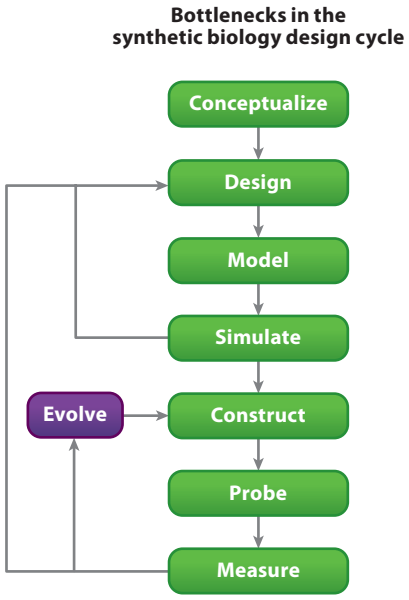


Figure 1

Design cycle for engineering circuits in synthetic biology. Although circuit conceptualization, design, and construction have made major progress, the field still faces bottlenecks at various phases along the entire cycle, including modeling, simulation, probing, and measurement.

Although many exciting advancements have been made, individual steps in the biological design cycle require significant improvements. For example, our ability to model the stochastic and dynamic behavior of synthetic designs is limited to small-scale circuits. Ellis et al. (1) demonstrated that detailed models coupled with diversified promoter libraries can guide predictive synthetic circuit design for straightforward feed-forward loops. However, using current techniques, researchers would need significant experimental data and computational resources to scale these models to account for all chassis-circuit interactions and larger circuit designs. New simulation platforms, such as those that can directly model biochemical reactions using the inherent physics of electrical hardware (2), may enhance the scale at which synthetic circuit models can be built. However, these large-scale models will still require detailed experimental data to achieve accuracy and utility. Owing to this lack of predictive models, synthetic biology projects are often carried out experimentally followed by post hoc modeling and simulations. Thus, novel computational architectures and a clear understanding of what levels of detail can be abstracted away are needed to achieve useful in silico design. Furthermore, a single laboratory may not contain all the expertise needed to develop and optimize all the steps in this design cycle; thus, efforts that facilitate cooperation will be crucial. Here, we endeavor to provide an updated review on recent advances in synthetic biology, focusing on selected steps in the biological design cycle. This review is intended to complement other recent reviews and shed perspectives on the current status of synthetic biology as an engineering discipline and its future prospects (3–7).

DNA CONSTRUCTION

Synthetic biology aims to build increasingly complex biological systems from standard interchangeable parts. Parts can be combined to create devices, multiple devices can form

higher-order systems, and systems can form large networks and ultimately synthetic chromosomes and genomes. A critical hurdle to achieving this goal is the physical assembly or synthesis of large DNA constructs that implement functional circuits.

The challenge in DNA assembly lies in creating a method that enables efficient, ordered assembly of DNA pieces. Traditional genetic engineering involves integrating a sequence into an expression vector using restriction-enzyme cloning sites. Although straightforward and practical for a low number of inserts, this method faces two limitations: (*a*) each unique insert to be integrated requires unique restriction sites, and (*b*) the restriction site sequences must be absent from the insert sequence. Because cloning a single piece of DNA requires a round of PCR amplification, restriction digest, gel purification, ligation, and transformation, the traditional technique becomes laborious with increasing circuit complexity. These limitations can become prohibitive when constructing large constructs such as metabolic pathways and complex regulatory circuits, which can involve more than a dozen separate parts and genes.

Therefore, in developing protocols for DNA assembly, synthetic biologists seek methods that are (*a*) sequence-independent, such that any DNA sequence can be assembled; (*b*) one-pot, allowing for all pieces, devices, and enzymes to coexist and react within the same vessel; (*c*) ordered, allowing the rational arrangement of pieces in a specific sequence; (*d*) capable of combinatorial assembly, allowing the swapping of multiple parts at a single site to generate large libraries of constructs; and (*e*) automatable. Many assembly methods addressing these needs have recently been developed and reviewed in detail (8). These include the BioBrick and BglBrick standards (9), sequence- and ligase-independent cloning (SLIC) (10), Gibson assembly (11), circular polymerase extension cloning (12), and golden gate assembly (13, 14). Although powerful, these assembly methods require careful planning and can be error-prone in the construction of large networks if they are manually performed. Automation algorithms and software packages facilitate the design and construction of circuits; examples include the Clotho framework and Eugene language for the BioBrick standard (15, 16) and j5 for Gibson/SLIC/golden gate assembly (**http://j5.jbei.org**). These tools can significantly reduce error rates and manual planning by optimizing assembly sequence and strategy, integrating combinatorial library design, and interfacing with robotics for automation. Software can thus allow synthetic biologists to focus on design and characterization rather than on specific assembly protocols.

DNA synthesis opens the possibility of a world in which circuits are directly designed and manufactured without requiring assembly from multiple parts (17, 18). Although productivity continues to rise and costs continue to decline, it is not yet feasible to synthesize large combinatorial libraries of synthetic circuits. For now, synthesis may be primarily appropriate for generating basic network topologies and optimized genes that can be subsequently tuned and diversified using mutagenesis and/or DNA assembly techniques. As synthesis capabilities increase and circuit designers become better at predicting and tuning circuit function in silico, DNA synthesis will be a valuable complement to DNA assembly and supplant it in certain applications. We expect that the eventual structure for biological circuit design will be analogous to electrical circuit design, wherein specialized foundries implement the physical construction of circuits created by "fabless" circuit designers. This specialization of labor has greatly facilitated the design and construction of complex electronic systems and should translate to biological systems as well.

BASIC PARTS

Basic biological parts are the building blocks of higher-order synthetic circuits. They are entities that perform specific functions such as regulating transcription, regulating translation, binding small molecules, binding protein domains, and modifying genetic material. Most parts originate

from nature; synthetic biologists co-opt these parts and engineer them for use in applications outside of their natural settings. A classic example is the use of natural promoters such as P_L of phage lambda and the *lac* operon promoter to control gene expression in prokaryotes (19, 20). Limitations in quantitative control and range of regulation led engineers to modify binding sequences for activators and repressors, giving rise to new promoters that enable tighter control and orders-of-magnitude greater ranges of regulation (21, 22).

This nature-to-synthetic approach has been applied to create a diverse library of parts, including RNA riboswitches (23, 24), RNase III domains (25–27) (**Figure 2*a***), microRNA (miRNA) (28), antisense-RNA transcription attenuators (29), ribosome binding sites (30), zinc fingers (31, 32), TAL effectors (33, 34) (**Figure 2*b***), DNA recombinases (35–37), LOV photosensors (38), and protein-protein interaction domains (39, 40). Synthetic biologists have enhanced the capabilities of these parts in various ways, including altering recognition specificity (23, 33, 37, 40, 41), enabling control with small molecules (26, 28), expanding the range of activity to allow tuning (27, 30), creating independently functioning variants (29), enabling activity in novel conditions (24, 36), and achieving enzymatic activity through protein fusions (31, 32, 34, 38, 42).

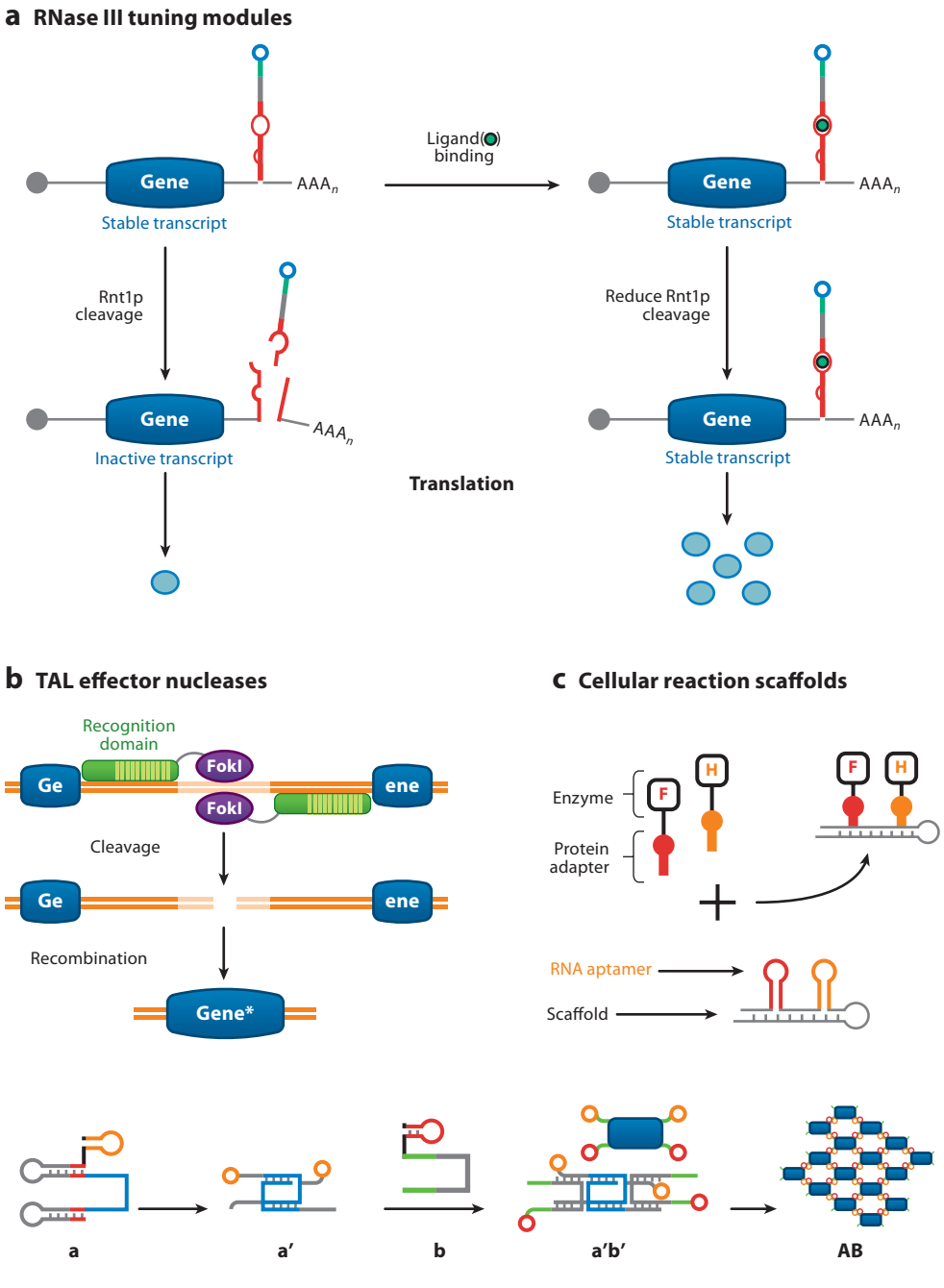
Novel parts can also arise from rational design, which requires predicting the influence of genetic changes on part function. Thus far, most such work has occurred with nucleic acids, which generate predictable base pairing and secondary structures (43, 44). For example, Delebecque et al. (45) recently designed RNA scaffolds to organize intracellular reactions spatially. Rationally engineered RNA modules self-assemble into one-dimensional and two-dimensional scaffolds and display protein-docking sites (**Figure 2*c***). By spatially patterning enzymes involved in hydrogen production, the authors increased H_2 production by 48-fold, showing the utility of spatial organization in a biosynthetic pathway.

Directed evolution is a powerful method to engineer new parts without detailed a priori insight into the specific modifications needed to achieve new activity (37, 46). However, a single round of mutation, screening, and selection can require days and frequent human intervention using conventional means. Two recently reported automated evolution approaches allow significant reductions in cycle times and human effort. Wang et al. (47) developed multiplex automated genome engineering (MAGE), a method of generating substantial sequence diversity on a genome-wide scale. The system involves automated control of cycles of cell growth, diversity generation, and recovery (**Figure 3*a***). Diversity is generated by inducing bacteriophage single-stranded DNA-binding protein β, which allows integration of oligonucleotides into replicating DNA. The authors sought to optimize the lycopene production pathway by introducing degenerate oligonucleotides at ribosome binding site sequences for each of the 20 genes and by inactivating 4 genes from secondary pathways. As many as 15 billion variants were generated, yielding a strain demonstrating a fivefold increase in lycopene yield. Depending on the oligonucleotides added to the pool, MAGE can be scaled to evolve single parts to large networks and the entire genome.

Esvelt et al. (48) reported a system for continuous directed evolution wherein 24–30 rounds of evolution occur per day with minimal human intervention. The phage-assisted continuous evolution (PACE) system directly couples the desired activity of the evolving protein to infectivity of phage particles (**Figure 3*b***). Theoretically, any protein activity that can be coupled to production of pIII can be evolved via PACE, including polymerases, protein-protein interactions, and recombinase activity. Similarly, if MAGE were adapted to integrate a selection step into the automated cycles, it could also become a continuous-evolution system.

An ever-expanding library of parts would enable synthetic biologists to build more complex networks. Discovery of more parts will likely arise from continuous mining of the literature and sequenced genomes for novel parts in diverse organisms as well as from an increasing ability to relate structure and function and thus achieve rational or combinatorial design of proteins and

nucleic acids. High-throughput implementation for these approaches will be crucial to establishing scalable-parts libraries. For example, pipelines can be developed to identify homologous parts in different hosts, optimize and synthesize the codons of these variants, and test them in suitable host chassis and frameworks.



CHARACTERIZATION

The growing library of synthetic biology parts enables the development of novel devices and networks. However, a deficit in the characterization of parts severely hampers the ability to design and model circuits to achieve predictable activity. As a result, circuit construction in synthetic biology has suffered from rudimentary design and laborious repetitions of trial and error. Ideally, parts collections should document the orthogonality of individual parts with each other and with the host chassis in which they have been characterized. This would allow them to be used simultaneously in a given cell and enhance our ability to model their behavior.

Standard Datasheets

A major contributor to this deficit in characterization has been a lack of clarity in what constitutes sufficient characterization for design and modeling. Datasheets for components in electrical and mechanical engineering include information on input/output characteristics, operating conditions, form factors, time dependence, and other details allowing the matching of compatible components for specific applications. Meaningful characterization of parts can be facilitated by a standard datasheet that, by general consensus, describes informative properties and standards for characterization of the part.

Canton et al. (49) proposed such a datasheet for a small-molecule-inducible transcription factor. Using GFP as an output, the authors characterized properties such as static performance, dynamic performance, activity through a range of inducer analogues, and performance reliability. The authors defined the output as the flow of RNA polymerases along a point of DNA per second (polymerase operations per second, or PoPS), a measure not directly detectable, but rather calculated from experimental data on protein and mRNA production and degradation rates. PoPS was chosen to act as a common input/output unit that can couple many parts together; for instance, a promoter generating a certain level of PoPS could predict the signal of a fluorescent protein placed under its control. This work represents an important effort to make order of the panoply of synthetic biology parts and will inspire additional studies into characterization parameters.

The dynamics, diversity, and unknowns of biology may require a plethora of additional details affecting performance at multiple levels—the part (e.g., robustness to mutation, performance at different temperatures), its host (e.g., growth stage of the population, activity in different cell types), method of characterization (e.g., precision, instrumentation), and interactions external to the part (e.g., orthogonality to host cell, orthogonality to other parts). The challenge is to balance utility, feasibility, and fungibility between different standards. For example, protein-protein interactions and transcriptional regulation require very different sets of characterization parameters.

Figure 2

Basic synthetic biology parts with novel functionality. (*a*) Rnt1p hairpin substrates in the 3′ UTR (untranslated region) of transcripts enable ligand-controlled cleavage. In the absence of ligand, Rnt1p cleaves the transcript and inhibits translation. Ligand binding inhibits Rnt1p activity and allows increased protein production. Modified with permission from Reference 25. (*b*) TAL effector nucleases are fusions of TAL effectors and endonucleases, such as FokI. TAL effectors bind to specific recognition sequences resulting in dimerized FokI domains that cut DNA and enable recombination and mutations to occur. Asterisk indicates new gene. Modified with permission from Reference 34. (*c*) Protein adapters are fused to ferredoxin (F) and hydrogenase (H) and can bind RNA aptamer sequences to yield programmable scaffolds. RNA strands a and b self-assemble into a′b′ and higher-order structures such as AB. Fixed proximity of enzymes F and H increase product yield. Modified with permission from Reference 45.

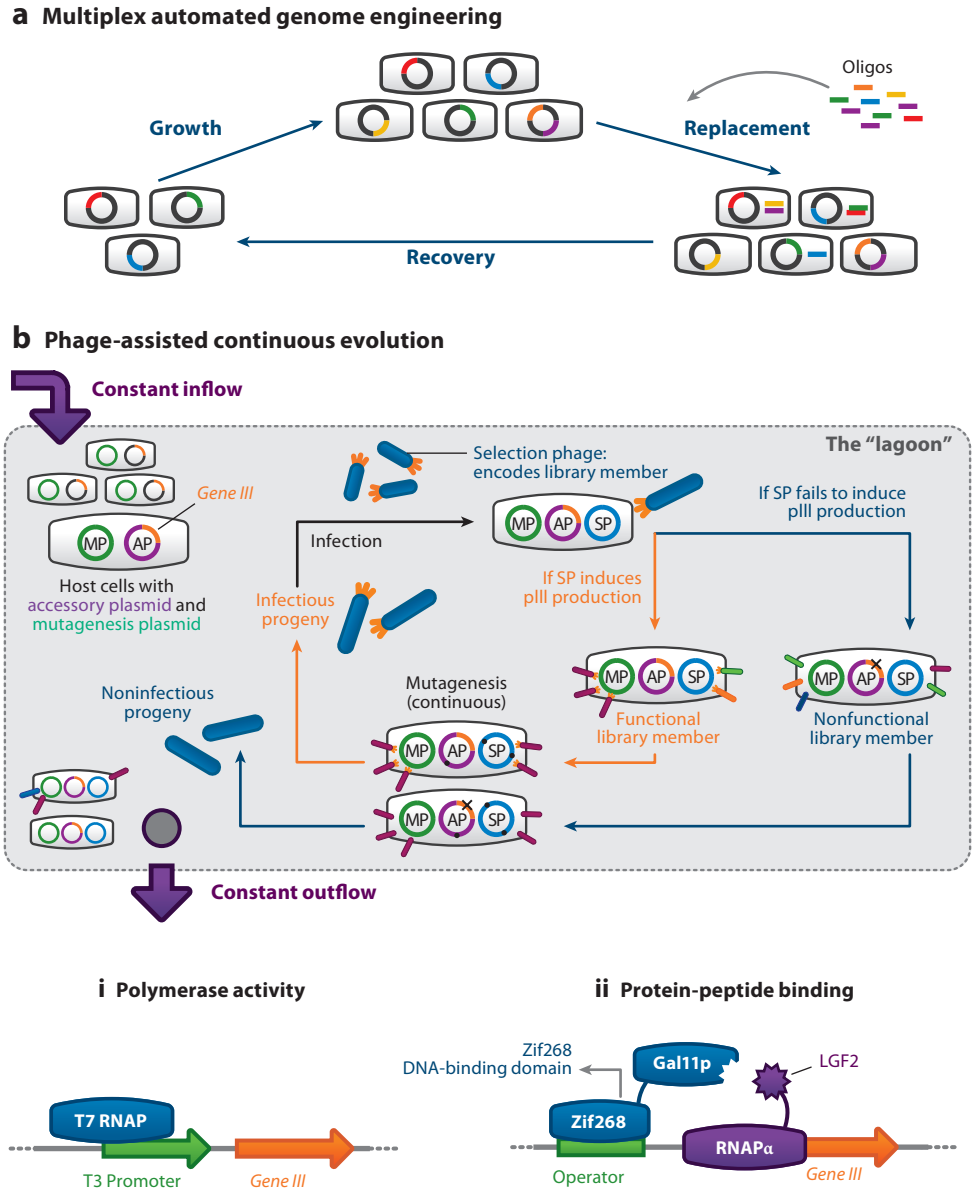


Figure 3

Facilitation of evolution to diversify new parts and genomes. (*a*) Multiplex automated genome engineering automates cycles of diversity generation, recovery, and growth. Diversity is generated by incorporation of oligonucleotides during genome replication. Modified with permission from Reference 47. (*b*) Phage-assisted continuous evolution ties the evolution of a target molecule to phage propagation to remove the need for human interventions. Each phage contains a selection plasmid (SP) encoding a library member. Host cells contain an accessory plasmid (AP) encoding *gene III*, which is critical for phage production, and a mutagenesis plasmid (MP), which creates diversity via mutation. Linking library-member performance to the production of phage protein pIII allows the propagation of successful phages to persist within the lagoon. pIII production can be tied to many protein activities, including polymerase binding to promoter sequences and protein-protein binding. Modified with permission from Reference 48.

The protein-protein interaction needs to account for parameters such as binding affinities, kinetics, and enzymatic activities (50). Moreover, characterization is intimately tied with the concepts of abstraction and modularity. If a given part behaves very differently depending on the circuit and cellular contexts it operates within, the utility of a datasheet that is developed in a limited set of conditions is diminished. Addressing this problem will require devices that perform buffering between circuit components, systems biology studies to capture the effects of chassis on circuits (and vice versa), and parts that are explicitly designed to minimize cross talk (51).

Standardizing Measurement

Even with a reliable datasheet composition, experimental conditions vary widely between—and even within—laboratories such that two identical parts studied in two different settings may yield divergent results. An analysis of 80 papers that used β-galactosidase as a measure of gene expression showed that the various authors used at least six different protocols with differences in substrates, experimental conditions, and output units (52). These variations pose a great challenge to characterization.

One method to counteract this is to characterize part activity relative to a standard reference object. Kelly et al. (53) applied this strategy to promoters by selecting one promoter to act as a standard and requesting multiple laboratories to characterize a set of promoters relative to the standard. Despite lack of standardization in protocols, the labs generated less than a twofold variation between described promoter activities. The authors then created a standard promoter-measurement kit with the reference standard for distribution to any lab wishing to characterize promoters. Scaling this approach for all parts would require the selection of a robust standard for each separate part category. The integration of such references into a set of standardized host chassis would be a useful step to encourage their more widespread use.

The Future of Characterization

The rapid evolution of synthetic biology poses a challenge to constructing a set of perennial standards that can avoid obsolescence. For example, DNA assembly techniques and standards have undergone multiple improvements within several years of being described and may be ultimately supplanted by DNA synthesis. This can be troubling for groups who have dedicated significant effort to curating numerous parts that conform to a defined standard. Even with secure standards in place, a single academic group often has few incentives to undertake the considerable investment to characterize a part reliably, as this primarily benefits external users.

Datasheets for components in other engineering disciplines are typically created by manufacturers who are strongly incentivized to characterize their parts for sale. A similar industry for biological parts may be required to achieve comparable levels of rigor. However, demand for biological parts will arise primarily when engineers are able to construct useful and commercializable combinations of biological parts—which, in turn, requires sufficient characterization. This cyclical relationship poses a challenge for ramping up characterization efforts. Thus, much of the characterization effort has resided in organizations such as BIOFAB, which was founded with the pioneering goal of creating open technology platforms to support the design, construction, and characterization of thousands of biological parts.

Finally, although it may be feasible to have characterization adhere to standard datasheets and to develop measurement standards for single parts, combinatorial circuits create exponentially more constructs that must be characterized. Thus, just as assembly techniques have enabled combinatorial circuit construction, the development of high-throughput characterization and

cataloguing techniques will be needed to harness the potential of an ever-expanding body of devices and circuits.

INTERFACES

Multiplexed inputs and outputs are important for tuning, probing, and characterizing synthetic biological systems. In particular, the dynamic and stochastic behavior of synthetic circuits must be characterized to ensure their proper operation. Diverse modalities have been adapted as inputs to biological circuits, including chemicals and light (54–57). The field still needs larger libraries of orthogonal inputs that can be used to manipulate multiple nodes simultaneously within biological systems. These libraries are critical to the biological design cycle, as they directly impact the throughput and accuracy with which synthetic systems can be tuned and characterized.

Chemical inducers are the primary mode for controlling biological inputs, but only a few are in routine use. Platforms for identifying and engineering chemical-ligand-triggered proteins and RNA parts have been described and need to be expanded (41, 58). To trigger cellular inputs, light is a useful modality that is orthogonal to chemical inducers. For example, bacteria have been engineered to switch states by light exposure (54) and detect light-dark edges (57). Other potential input modalities may involve the use of magnetic transduction (59, 60) or mechanical forces (61). Small RNAs that mediate RNA interference may be useful as scalable and orthogonal inputs if efficient delivery vectors can be developed (62).

One of the keys to debugging complex systems, such as electronic chips or computer programs, lies in the ability to record outputs from multiple internal nodes. In biology, monitoring the outputs of synthetic circuits relies largely on fluorescent proteins (63). On-line measurements of gene expression using electrochemical sensors to detect *lacZ* expression have also been described (64). However, their multiplexing capabilities are relatively limited; thus, techniques for scalable biological outputs are needed. Systems biology technologies are well suited for these studies as they are able to capture genomic-, transcriptomic-, and proteomic-level data (65, 66). By simultaneously measuring the state of the host chassis and the behavior of the synthetic circuit, such techniques can help construct more accurate models of circuit performance. However, these technologies are currently too expensive to be used for routine, time-lapse measurements in multiple experimental conditions, which are needed to characterize synthetic circuits.

HIGHER-ORDER CIRCUITS AND PLATFORMS

Creating programmable functionality from synthetic gene circuits is one of the ultimate goals of synthetic biology. Synthetic biologists have constructed a host of higher-order devices from basic parts, including analogues of electrical engineering components such as switches and memory elements (67–73), cascades (74), pulse generators (75), time-delayed circuits (76, 77), oscillators (68, 78–81), filters (82), and logic gates and processors (83–86). Here, we highlight a few recent examples.

Working on cellular control, Callura et al. (87) reported a riboregulator-based bacterial kill switch. By placing two phage lysis genes under control of different riboregulator variants, the authors constructed an AND gate requiring three separate small molecules to lyse the cell (**Figure 4*a***). The switch construct allows for tight control, fast response time, and minimal leakage, preferred qualities in a kill switch that might be used in a bacterial delivery vehicle.

In addition to synthetic circuits implemented within single cells, multicellular computation has been achieved using quorum sensing and cell-cell communication modules. For example, complete two-input logic functions have been constructed by constraining each cell to express just one logic

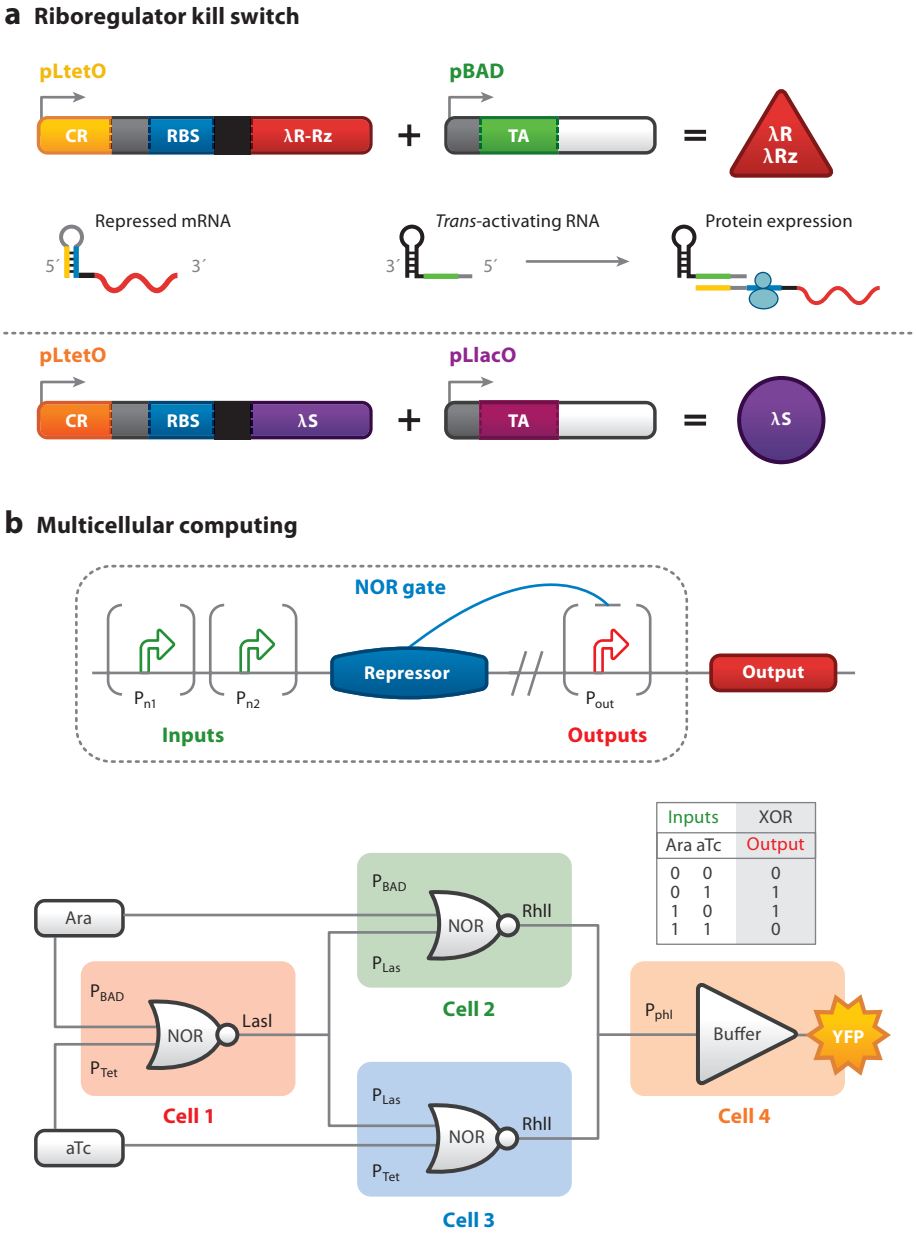


Figure 4

Higher-order circuits integrate multiple parts. (*a*) In this RNA-based riboregulator, the *cis*-repressive sequence (CR) binds the ribosome binding sequence (RBS) to inhibit translation. In the presence of *trans*-activating RNA, the *trans*-activating sequence (TA) binds the CR and allows the ribosome to bind RBS and initiate translation. Two lysis proteins are put under control of three small molecules, allowing tight control of this kill switch. Modified with permission from Reference 87. (*b*) A NOR gate produces output only when neither input is present. Spatial positioning of NOR-gate-containing cells with different inputs and outputs creates the emergent multicellular XOR gate. Other positioning patterns allow construction of all 16 two-input Boolean logic gates. Modified with permission from Reference 88.

gate (88, 89), thus circumventing the challenge of encoding multiple logic gates within a single cell (**Figure 4*b***).

Ideally, modular genetic circuits can be assembled and swapped to create a desired function in defined test organisms, then introduced with intact function into more complex cellular systems. In current practice, the predictability of synthetic circuit design is hampered by various failure modes, including those internal to the circuit (incompatibility of input and output; interaction between parts, noise, and mutations) and external (cellular context dependence, extracellular environments, and unknown interactions with host components). As a result, engineering paradigms for constructing genetic circuits have included a trial-and-error approach using swappable modular parts, directed evolution, or a combination of both (90). Computational and experimental techniques that explicitly account for these failure modes are necessary to advance the current practice of synthetic biology (91). For example, in addition to novel functionalities and behaviors, redundancies and failure mechanisms should be incorporated into synthetic circuits to ensure robust functions. Test platforms where global parameters and interactions are known and can be tuned will also assist in the engineering of complex synthetic gene networks (3).

DIRECTED APPLICATIONS

Advances in parts and circuit design not only allow progress toward the lofty goal of programmable cells but also enable the current development of targeted applications. We illustrate a few broad categories as examples where synthetic biology has been successfully applied to enable new functionalities.

Metabolic Engineering

The boundaries and overlap between metabolic engineering and synthetic biology are often blurry as practitioners often work in both fields, which also share common tools (92). In general, we suggest that metabolic engineering entails constructing and optimizing biosynthetic pathways to maximize yields of desired products. Applying synthetic biology principles and tools enables enhanced outputs, including integration of novel pathways, tuning of pathway flux, control over production, and the ability to create new outputs using biomanufacturing.

Microbial production of biofuels and high-value chemicals has been a focus of recent excitement in metabolic engineering for economic and environmental reasons, with production methods ranging from converting biomass (93) to harnessing photosynthesis (94). Advances in synthetic biology have allowed the transference of metabolic pathways into non-native hosts that are suitable for industrial bioprocesses. Genetic engineering has enabled corresponding improvements in biofuel tolerance (95), the expansion of the range of carbon source inputs (96), and enhanced yields (97). Similar approaches have been applied to a host of desirable products, including artemisinic acid (98), polyketides (99), non-natural amino acids (100), and others reviewed previously (101–103). Strategies for pathway expression and optimization include whole-operon synthesis with codon optimization (104), tuning of transcription rates via promoter libraries (105), tuning of translation rates via ribosome-binding sites (30), physical scaffolds for enzymes (45, 106), and directed evolution (47, 107).

Biomedicine

Synthetic biology has the potential to engineer novel diagnostic and therapeutic strategies for relatively intractable medical conditions such as cancer and infectious diseases. Realizing the

promise of synthetic biology for biomedicine will require significant efforts to characterize the reliability, adverse effects, and in vivo performance of engineered circuits and cells. An additional challenge to implementation in the clinic is efficient delivery of engineered cells or artificial gene constructs to endogenous cells. Ongoing clinical trials in stem-cell therapy and gene therapy will provide useful technological and regulatory precedents for more complex synthetic biology approaches to treating diseases. Clinical applications have recently been reviewed (6); thus, we highlight only a few applications relevant to biomedicine.

Cancer. A critical shortcoming in cancer treatments has been their inability to distinguish between cancerous and normal cells. One reliable signature of tumor growth is hypoxia; by employing heterologous sensors, Anderson et al. (108) engineered *Escherichia coli* to invade mammalian cells selectively in hypoxic environments. Recently, Wright et al. (109) employed similar principles to link enzymatic activity to a cancer marker of hypoxia. HIF-1 a is a hypoxia-inducible factor selectively found in cancer cells. The authors coupled the activity of a segment from p300, a binding partner of HIF-1 a, to the activity of cytosine deaminase, an enzyme that converts the relatively benign prodrug 5-fluorocytosine to the chemotherapeutic 5-fluorouracil. This enabled selective activity of the drug within cancer cells, which could result in significant improvement in the side effects typical of chemotherapy.

Xie et al. (110) recently described a miRNA-detection strategy that enables the selective identification of cell type by miRNA expression signature. Individual circuits can selectively identify if a given miRNA is expressed at either high or low levels; the combination of these circuits creates a cell-type classifier triggering a response if miRNA levels match the profile of interest (**Figure 5**). The authors selectively detected and killed HeLa cancer cells by putting the expression of a proapoptosis protein under conditional control by two high and three low miRNAs. One can envision interfacing such circuits with other biomedical modalities to achieve enhanced diagnosis, imaging, and treatment of difficult medical diseases for which unique miRNA signatures can be identified.

Infectious disease. The rise of antibiotic resistance and properties such as biofilm formation (111) and persistence (112) have made microbial infections increasingly difficult to treat. The rapid development of antibiotic resistance in pathogens often necessitates treatment with potent antibiotics. This is problematic owing to the dearth of new antibiotics being discovered and translated into clinical use. Furthermore, antibiotics can generate undesired and significant perturbations in the human microbiome by non-specifically killing non-pathogenic bacteria. Selective targeting of pathogens using synthetic biology or other strategies may avoid this side effect. Synthetic biology also enables the design of new treatment methods to target bacterial biofilms (113), potentiate current antibiotics (114), and engineer new treatment vehicles.

Saeidi et al. (115) engineered *E. coli* to sense *Pseudomonas aeruginosa*, a bacterium causing infections in the lung, urinary tract, gastrointestinal tract, and skin that are refractory to treatment. The authors linked quorum sensing to expression of genes for pyocin, a bacteriocin, and E7, a lysis protein. Grown in the presence of *P. aeruginosa*, the engineered *E. coli* accumulated intracellular pyocin and E7; when sufficient levels of E7 lysed the cell, pyocin release killed the pathogen and inhibited formation of biofilm. The method illustrates a treatment vehicle that can recognize pathogens and release an antimicrobial locally. However, the introduction of any live bacteria into patients already suffering from infections will require extensive clinical testing and perhaps the ability to command the engineered cells to self-destruct.

Lu & Collins have described two strategies in which engineered phages can be used to target bacterial biofilms and antibiotic-resistant bacteria, raising the possibility that synthetic biology can

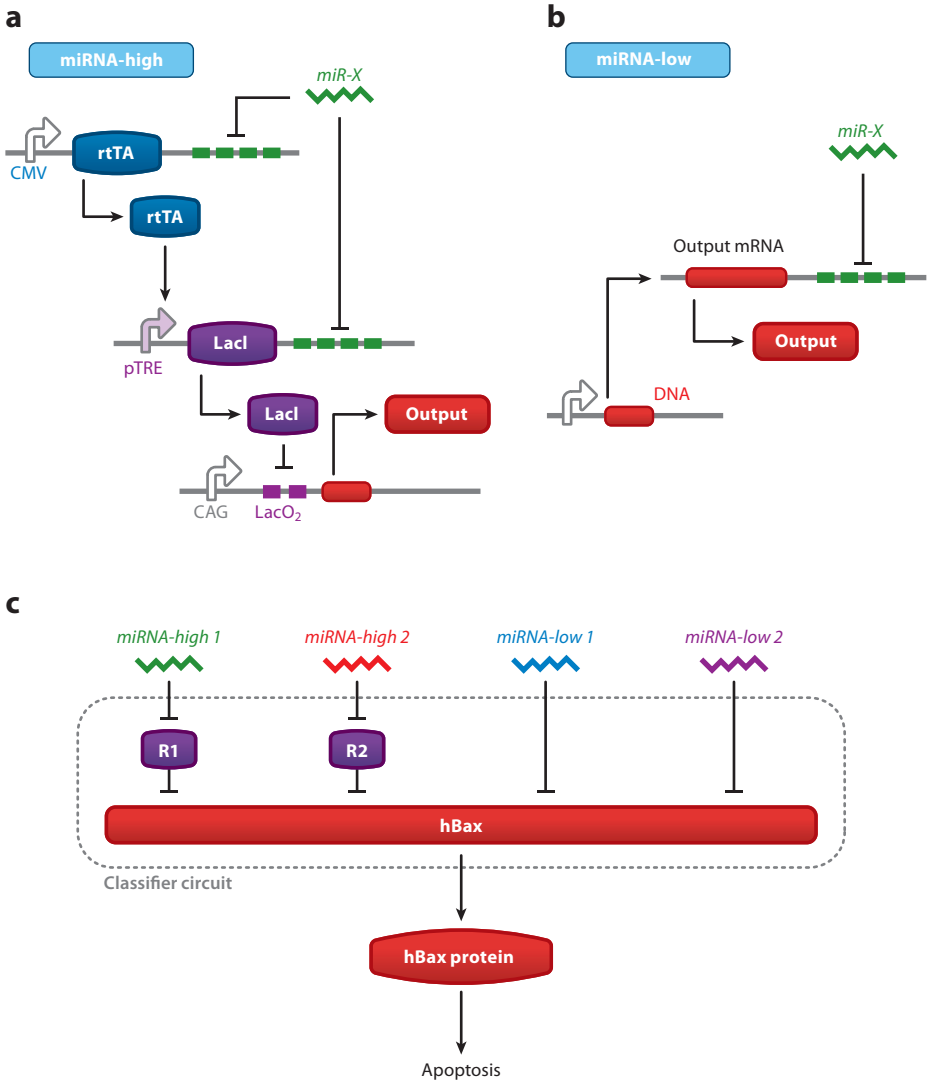


Figure 5

Cell-classifier circuit based on integrated microRNA (miRNA)-level detection. (*a*) High-miRNA sensor. High levels of miRNA lower levels of rtTA and LacI, allowing expression of the output protein. Low levels of miRNA allow high levels of LacI, which represses output. (*b*) Low-miRNA sensor. Low levels of miRNA allow translation of output messenger RNA (mRNA), whereas high levels of miRNA allow RNA interference (RNAi)-mediated destruction of the mRNA. (*c*) The integration of multiple high and low sensors yields a classifier circuit. The resulting output of a proapoptotic protein allows for the selective killing of cancer cells. Modified with permission from Reference 110.

yield a more sustainable source of antimicrobial agents to combat the ever-rising tide of microbial pathogens (113, 114, 116). In the first strategy, phages were designed to force the expression of biofilm-degrading enzymes during infection of bacterial cells, resulting in a positive-feedback loop involving the degradation of biofilms and amplification of phages (113). In the second strategy, phages were engineered to express suppressors of networks implicated in antibiotic resistance

and used to enhance the efficacy of coapplied antibiotics (114). Owing to the natural diversity of phages and enhanced engineering platforms, these strategies may enable the development of antimicrobial pipelines that can keep pace with the evolution of resistance in pathogens.

Autoimmune disorders. Autoantigen recognition by the human immune system leads to the development of a number of autoimmune diseases, including lupus, multiple sclerosis, and type I diabetes. However, the inciting autoantigens are often unknown, and attempts to find them have traditionally covered a small fraction of all potential targets. To probe the entire coding region of the human genome, Larman et al. (117) constructed a library of phages displaying 36-residue peptides spanning all open reading frames in the genome. The authors then used immunoprecipitation sequencing with cerebrospinal fluid from patients suffering from autoimmune disease of the central nervous system and identified novel potential autoantigens and autoantibodies. This is an effective method enabling discovery of a broad range of autoantigens as well as generic peptide-protein interactions. Though not currently leveraging the regulatory circuits of synthetic biology, such studies can thoroughly benefit from the high-throughput DNA synthesis and assembly techniques described above.

Engineering Genomes

Synthetic biology in its most literal sense can include the creation of artificial life within the laboratory and efforts to uncover the origins of life on our planet (118, 119). A more tractable challenge today is the engineering of existing genomes for biomanufacturing or to decipher the principles that govern the operation of biological systems (120). By employing a version of MAGE staggered over several hierarchical steps, Isaacs et al. (121) developed conjugative assembly genome engineering to replace all 314 TAG stop codons in the *E. coli* genome with TAA codons. The authors divided the task into 32 parallel steps for efficiency and to avoid secondary mutation accumulation. Strains with the full replacement set survived after deletion of *RF1*, the sole release factor recognizing TAG. This work demonstrates the ability to generate genome-wide modifications, treating the genome as a rewriteable template.

The rapid improvements in DNA synthesis and enhanced assembly techniques enable the construction of entire genomes from scratch. Within several years, synthesis capabilities have progressed from a *Mycoplasma* genome of 582,970 base pairs (122) to a 1.08-mega-base-pair *Mycoplasma* genome transplanted into a recipient cell lacking a genome (123). Recently Dymond et al. (124) reported the remarkable synthesis of the right arm of chromosome IX in yeast and a portion of chromosome VI. Although the sequences are relatively short (90,000 and 30,000 base pairs, respectively), they are the largest pieces of eukaryotic DNA synthesized. More notably, these genomes were integrated into yeast cells with minimal phenotypic variation in growth and gene expression. The authors also made critical changes to the synthetic pieces, including (*a*) removal of 20 nonfunctional or repetitive sequences; (*b*) addition of watermarks to gene sequences; and (*c*) addition of recombinase sites after each nonessential gene, allowing for the generation of a library of genomic rearrangement variants. The work provides a valuable method of studying the yeast genome and adapting yeast to specific applications such as biosynthesis. It is only a matter of time before the approach is applied to the whole yeast genome and higher-level organisms, including humans.

CONTEXT WITHIN BIOMEDICAL ENGINEERING

By enabling the detailed control and rapid engineering of cellular processes, ranging from genetics to metabolism to cell-cell interactions, synthetic biology has the potential to be well integrated

with other efforts in biomedical engineering. The nature of these efforts may involve the use of synthetic regulatory circuits as intracellular sensing and control elements that interface with intracellular, extracellular, and abiotic systems.

Front-End Sensors

In many disease conditions, a real-time sensor inside or outside the body would greatly facilitate treatment decisions and detection of status changes in health. Panizzi et al. (125) developed a system to detect *Staphylococcus aureus* endocarditis in vivo via positron emission tomography. Bacterial colonization of heart valves requires aggregation of fibrin and platelets. The authors fluorescently tagged a prothrombin analogue and injected it into the mouse, where it was processed by *S. aureus* staphylocoagulase and incorporated into growing vegetations. The aggregated fluorescence was detectable by noninvasive imaging. The method provides a novel method for detection of a traditionally difficult-to-diagnose condition. Although such technologies do not currently involve living cells, one can envision developing more complex front-end sensors in engineered cells to report on important in vivo conditions, such as hypoxia in cancer, reactive oxygen species in inflammatory conditions, and quorum-sensing molecules in bacterial infections. With a more sophisticated ability to build networks predictably, engineers can develop diagnostic tools and engineer actuators triggered by sensors—for instance, the release of chemotherapeutics upon binding to tumor cells.

Tissue Engineering and Regenerative Medicine

An area where synthetic biology and biomedical engineering has the potential to be synergistic is tissue engineering. In the past several decades, tissue engineers have made tremendous strides in creating artificial organs and tissues utilizing strategies such as artificial scaffolds, cellular patterning, and extracellular signaling (126). Combining these efforts with the ability to manipulate intracellular control networks may enable significant breakthroughs in our ability to create complex tissues in a well-controlled fashion. However, for most organ systems, many critical challenges remain before engineers can create fully functional organs for transplantation.

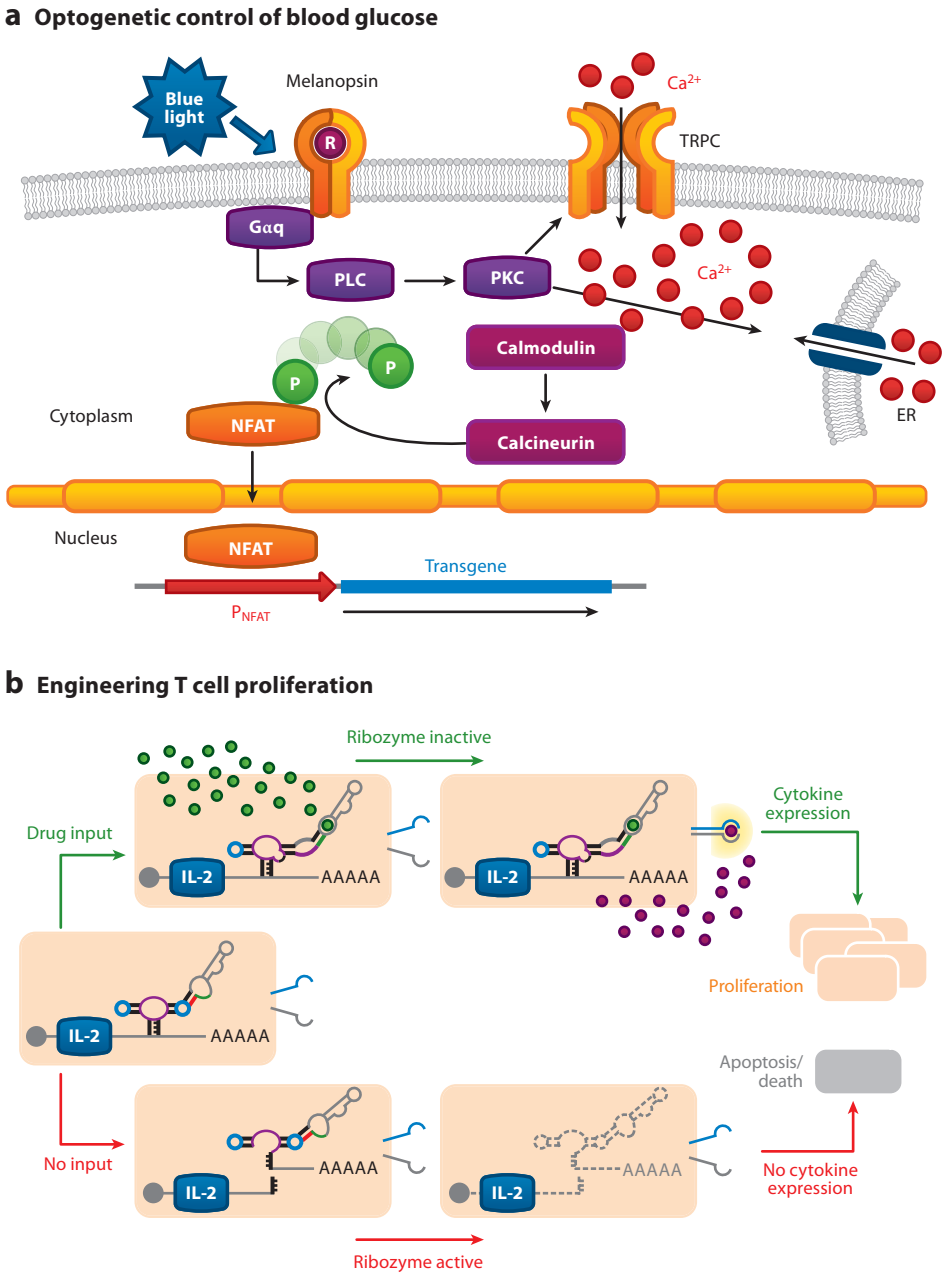
Replicating natural cell function in an artificial context is a central obstacle in regenerative medicine. Although this may be achieved by recapitulating the developmental pathway of the desired cell, developing a synthetic analogue of the natural cell may also be useful. Ye et al. (127) developed a light-controlled transgenic cell system to curb hyperglycemia in mouse models of diabetes. The authors coupled the melanopsin signal transduction cascade to a Ca^{2+}-dependent nuclear factor pathway (**Figure 6*a***). Coupled with devices that emit controlled pulses of light,

Figure 6

Applications of synthetic biology in biomedical engineering. (*a*) Blue-light activation of melanopsin initiates a pathway leading to intracellular calcium increase. Subsequent activation of calmodulin enables translocation of the transcription factor NFAT into the nucleus and transcription of a transgene. Putting glucagon-like peptide-1 under NFAT promoter control and implantation of transgenic cells containing this circuit enables light-controlled blood glucose homeostasis. Modified with permission from Reference 127. (*b*) A ligand-controlled ribozyme in the 3′ UTR of the cytokine IL-2 achieves regulation of cellular proliferation. In the absence of drug input, the 3′ UTR ribozyme is active and cleaves the mitochondrial RNA, causing destabilization, lack of cytokine expression, and apoptosis of the cell. Drug input inactivates the ribozyme, allowing translation of IL-2 and cell proliferation. Abbreviations: ER, endoplasmic reticulum; IL, interleukin; P, phosphate; PKC, phosphokinase C; PLC, phospholipase C; TRPC, transient receptor potential channels; UTR, untranslated region. Modified with permission from Reference 128.

these cells can be triggered to express cellular factors such as glucagon-like peptide 1 to attenuate glycemic excursions and simulate responses of pancreatic β-cells.

Rational control over developmental pathways and cell growth would allow tissue engineers to grow specific cell types along a predetermined timeline. Chen et al. (128) engineered a ribozyme-based translational control system to modulate human T-cell proliferation. By integrating a theophylline-responsive ribozyme into the 3′ UTR (untranslated region) of the cytokine



interleukin-2, the authors demonstrated precise and tunable control over T cell growth and division (**Figure 6*b***). Coupling similar inducible controls to genes determining differentiation and cell growth would allow fine control over the fate of cells.

In addition to the identity of cells, the spatial arrangement of cells is critical to function in many organs. Basu et al. (75, 129) co-opted bacterial quorum-sensing systems to build programmable pattern formation in shapes such as ellipses and clovers. Recent work by Sprinzak et al. (130, 131), elucidated the mechanism behind the Notch-Delta developmental signaling pathway. Delta in one cell can bind to and activate Notch in a neighboring cell; however, Delta inhibits Notch activity in its own cell. This behavior leads to formation of sharp boundaries and lateral inhibition between cells. Patterning systems based on this behavior would allow precise spatial control of cellular patterning at the single-cell level.

Clearly, much biology in developmental pathways and pattern formation needs to be elucidated before most organs can be reliably engineered to be functional within the body. However, synthetic biology methods will develop in parallel to discovery efforts to achieve the ultimate goal of tissue engineering.

Biomaterials

Synthetic biology may enable the discovery of novel biomaterials and the cell-based synthesis of useful biomaterials. The ability to manipulate genetically the sequence and structure of biomaterials enables both rational and evolutionary strategies to be applied. Furthermore, the diversity of biological processes is a large source of new biomaterials with properties that can outperform synthetic materials. For example, compared with Teflon, bacterial biofilms have greater resistance to wetting by water (132). Widmaier et al. (133) engineered the *Salmonella* type III secretion system along with codon-optimized versions of silk proteins to create a microbial silk-production system. DNA nanotechnology enables the accurate construction of in vitro nanopatterns that can serve as scaffolds for biomaterials (134, 135). These capabilities may yield new scaffolds for tissue engineering, enhanced surgical materials, and biocompatible device coatings for medical applications.

CONCLUSIONS

The field of synthetic biology has covered much ground in the past decade but remains an emerging area of research. Significant challenges lie ahead on its path toward a rational engineering discipline that will enable important biomedical applications. Scientific efforts that result in broadly useful platforms for the broader community are needed to complement application-specific thrusts that may be less generalizable. With new tools and approaches, synthetic biology may be well poised to advance novel therapeutic modalities, diagnostic tools, and scientific methodologies to help researchers understand biological systems in health and in disease.

FUTURE ISSUES

1. High-level design tools coupled with rapid and inexpensive DNA synthesis and assembly technologies will accelerate the prototyping, tuning, and deployment of synthetic biological systems for applications.
2. Predictive computational models that are validated by experimentation and applicable across many host organisms and laboratories need to be developed.

3. The synthetic parts library should be enhanced with orthogonal and well-characterized components. Researchers need to develop platforms for the scalable generation of synthetic parts, mutagenesis of existing parts, and identification of novel parts from natural systems.
4. Defining the extent of biological modularity and cross talk will enhance our ability to build increasingly complex systems, understand potential interaction modes, and implement biological circuits across a variety of organisms.
5. Characterizing the long-term performance, behavior, stability, and fate of synthetic circuits will be necessary as the field moves closer to deployed applications.
6. Advancements in the fundamental tools of synthetic biology will be accelerated and guided by important biomedical applications, such as the diagnosis and treatment of cancer and infectious diseases. Collaborative efforts between synthetic biologists, biomedical engineers, and clinicians will be important in these avenues of research.
7. Synthetic biologists should continue to engage in outreach, education, and communication efforts to ensure the responsible advancement of synthetic biology technologies, enhance palatability with regulatory agencies, and secure public support.

DISCLOSURE STATEMENT

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