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Synthetic biology: exploring and exploiting genetic modularity through the design of novel biological networks†

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Synthetic biology has been used to describe many biological endeavors over the past thirty years—from designing enzymes and in vitro systems, to manipulating existing metabolisms and gene expression, to creating entirely synthetic replicating life forms. What separates the current incarnation of synthetic biology from the recombinant DNA technology or metabolic engineering of the past is an emphasis on principles from engineering such as modularity, standardization, and rigorously predictive models. As such, synthetic biology represents a new paradigm for learning about and using biological molecules and data, with applications in basic science, biotechnology, and medicine. This review covers the canonical examples as well as some recent advances in synthetic biology in terms of what we know and what we can learn about the networks underlying biology, and how this endeavor may shape our understanding of living systems.

Introduction

The complexity of biological systems emerges from the interaction between modular components in the form of small molecules, DNA, RNA, and proteins. As we learn more about these biological parts and the relationships between them, we are able to manipulate and recombine biological modules in live cells in a way that allows us to design novel biological pathways with useful behaviors. In the past decade, synthetic biology has also used language from computer science and engineering in order to separate itself from more traditional genetic engineering and recombinant DNA technologies that also rely on the power to manipulate cellular circuits. The extended analogy of cells as machines has been invaluable to developing the nascent field as well as for industrial applications of synthetic biology. This mirrors the development of computer science, where improving understanding of how evolved living systems work has shaped the development of computer technology, from early cybernetics to more recent work on genetic algorithms and neural networks. Designed and evolved systems will continue to affect each other’s development, especially in the coming age of designed cellular systems and genomes.

The comparison between biology and electrical engineering for synthetic biology begins with the concept of modularity. A system can be described as modular if its components can be functionally separated and recombined. Molecular biology is
highly modular, from its currently recognized fundamental units—nucleotides and amino acids—to its higher level organization in cells and organisms. The hierarchy of complexity in biology, from genes and proteins to biochemical reactions, pathways, cells and populations mirrors the hierarchy of computer engineering, which moves from transistors and other small physical components to logic gates, circuit board modules, whole computers, and computer networks (Fig. 1). Complexities may arise from the interactions between modules at different levels of organizational hierarchy, and in order to understand function at a given layer one can abstract the details at others. For example, in order to understand how to use a computer program one does not need to be able to understand the programming code it was written in, and in order to understand the function of a biochemical pathway, one does not need to know the physics of the interaction between the amino acids of each protein. This abstractability makes it possible to do reductionist experiments in biology, isolating specific modules for close study to eliminate variables introduced by the cellular context.

In synthetic biology, the simplification of biological systems into modular, abstracted networks allows for a bottom-up study of biology from first principles, in all layers of the biological hierarchy. The study of how genetic components work together to integrate environmental signals and effect a cellular response is a main focus of post-genomic biological research. Through synthetic biology, researchers are able to determine what topological arrangement of regulatory modules can lead to a given behavior, often with strong analogy to electronic signal processing units such as logic gates and feedback loops. These regulatory motifs can then be recombined with well-understood output modules for a

Fig. 1 Modularity throughout the biological hierarchy. (A) DNA sequences are built of nucleotides that encode different modular parts, including promoters, repressor binding sites, ribosome binding sites, introns with encoded miRNAs, exons, and terminators. (B) These DNA parts code for the expression of modular proteins in a tightly regulated manner. Proteins, such as human c-Src pictured here, often contain multiple domains that perform catalytic activity (kinase domain), mediate interaction with other proteins (SH2 and SH3 domains), and regulate their own activity (linker and autoregulatory tail). (C) Interactions between different proteins lead to complex behaviors of biological pathways locally, and globally throughout the cell. Synthetic biology is poised to recombine modules from all levels of hierarchy.
wide array of biomedical or industrial applications. This is true at a high level of abstraction in the attempts to make synthetic “protocells”, design the “minimal” genome, and synthetically explore embryonic differentiation. In this review, however, we focus on the design and implementation of synthetic networks at the level of transcription, translation, and signal transduction, where synthetic biology has had many successes in recent years.

Despite showing a sophisticated engineering of cellular processes, many call these engineered networks “toy” systems. While these projects are certainly on a small scale it is important to see them as valuable experiments towards a broader understanding of the principles that underlie biological networks and progress towards a more coherent effort to engineer cellular behaviors. Moreover, as these synthetic networks are built from modular, interchangeable components, the transition from toys to potentially useful technologies is a relatively easy one. The following examples of synthetic biology are intended to show how biology is currently being engineered and how these projects are shaping the future study of biology.

Engineering transcriptional regulation

Using genetic engineering technology, synthetic biologists can interact with multiple levels of biological complexity using similar, standardizable techniques. Because DNA sequences code for proteins as well as regulatory elements, synthetic biologists can design biological systems much in the same way as software engineers design computer systems. Complex biological programs can be built by stringing together well-characterized, modular pieces of code. Typically the modules being used by synthetic biology are natural DNA sequences taken from their natural context and recombined. These elements are versatile for a variety of applications and many have been used in genetic engineering for decades. Synthetic biology aims to turn commonly used transcription regulatory systems such as the Lac operon, the repressor system of lambda phage, and the Tn-10 encoded Escherichia coli tetracycline resistance (TetR) operon into standardized, well-quantified parts, much like the resistors and transistors of electrical engineering. The combination of standardization of parts and techniques with the diversity of biological output behaviors makes synthetic biology the powerful endeavor that it is.

Simple switches and epigenetic memory

Transcriptional regulatory networks are relatively well-understood biological systems that tightly control the expression of genes based on a multitude of environmental cues. Because of the long history of quantitative study and engineering of transcriptional regulation in E. coli, DNA based transcriptional regulation of synthetic networks has been a major component of synthetic biology research. Transcriptional regulation is modular, with transcription factors made of separable DNA binding and activator domains acting on modular promoters and/or repressors that can be tuned in strength and recombined to control arbitrary genes. Such engineered transcription networks both show how complex behaviors can emerge from interactions between simple genetic components and are important control systems for future engineered networks. Variants of the Lac, Tet, lambda, and other transcription control modules have been implemented in dozens of synthetic gene networks to create information processing feedback loops, logic gates, bistable switches, and/or oscillators. Combinations of these designed networks can be further assembled to develop increasingly complex genetic networks, mirroring the evolution of network complexity in cells. As simplified versions of natural transcriptional control mechanisms that function orthogonally to native cellular systems, these synthetic circuits provide insight into how gene connectivity can lead to a given behavior in their evolved counterparts.

One of the earliest and simplest gene networks studied synthetically is bistability, as exemplified by the toggle switch. The toggle switch represents a simple cellular epigenetic memory system, in which transient incubation with an activating small molecule will push the switch into a stable “on” state, which persists for many generations in the absence of the activator. This type of behavior is critical in a wide range of biological systems from bacterial gene regulation to development and cellular differentiation in metazoans. In cells, bistable switches are robust to transcriptional noise, requiring a stimulus above a certain threshold before activating. This noise-filtering behavior is likewise critical in synthetic circuits that need to behave predictably in a cellular context.

Strikingly, bistability can be engineered using modular components from E. coli regulatory elements that are not part of such complex behaviors in their native context. The design of the synthetic bacterial toggle switch simply involves two mutually inhibitory promoter–repressor pairs. Two stable states are possible in this system: either promoter A produces a molecule that represses promoter B, or promoter B produces the repressor for promoter A. The cells can be forced to switch between the two states by addition of an inducer that represses the activity of either one of the repressors (Fig. 2A).

Bistable systems such as the toggle switch are a generally applicable unit in building logic gates and are useful tools for biotechnology and basic science. For example, different input receptors can be interfaced with the toggle switch to produce inexpensive bio-sensors of dangerous pollutants such as TNT or arsenic. Engineered bistability can also be used in more complex synthetic systems through different combinations of input modules, leading to gates that activate in the presence of different combinations of two or more inducers. The epigenetic toggle switch has also been adapted to yeast, plants, and mammalian cells to tap into the increased behavioral repertoire of eukaryotes.

Epigenetic synthetic memory can also be achieved through other topological arrangements of transcriptional regulators. Ajo-Franklin et al. rationally designed a eukaryotic memory device in cells that retains memory of a transient change in nutritional availability based on positive feedback as opposed to the negative control of the toggle switch. Induction leads to transient expression of an activator that promotes transcription from a second promoter. Promoter B promotes production of its own activator, creating a positive feedback
molecules,27,28 environmental cues such as hypoxia,29 or even interactions,26 the ability to combine synthetic and natural orthogonal to existing biology to avoid complex cross-talk critical. Of genes in response to a variety of signals will be in gene therapy and tissue engineering, where tight artificial mammalian cell behavior in this way has potential application stimulus will affect the circuit. The ability to control A stimulus above a certain threshold will change how a future the state of the switch depends not only on the current characteristic of cellular signal processing networks, where biological systems. Changing the input module of a synthetic switch in mammalian cells.25 Hysteresis is a common architecture was used in implementing a hysteretic epigenetic switch in which the inducer molecule is added, promoter A is transiently activated, promoting expression of output 1 and activator B, which acts on promoter B. Activity of promoter B leads to expression of its own activator, a positive feedback loop that leads to heritable memory of the transient stimulus.24

loop that retains memory of the transient signal even as the cell divides (Fig. 2B). Similar positive feedback-based network architecture was used in implementing a hysteretic epigenetic switch in mammalian cells.25 Hysteresis is a common characteristic of cellular signal processing networks, where the state of the switch depends not only on the current conditions, but also on previous responses to stimuli. A stimulus above a certain threshold will change how a future stimulus will affect the circuit. The ability to control mammalian cell behavior in this way has potential application in gene therapy and tissue engineering, where tight artificial control of genes in response to a variety of signals will be critical.

While synthetic networks are typically designed to be orthogonal to existing biology to avoid complex cross-talk interactions,26 the ability to combine synthetic and natural genetic networks is a valuable tool for studying natural biological systems. Changing the input module of a synthetic memory switch from an arbitrary chemically inducible promoter to a promoter activated in response to biologically relevant signals such as DNA damage,27 quorum sensing molecules,27,28 environmental cues such as hypoxia,29 or even electrical current30 allow for the design of strains that can be used in the study of these important biological phenomena. Moreover, altering the switch output from simple fluorescence to any number of genetically controlled cellular behaviors can create useful biological machines. In a powerful example, bistable switch architecture was used in order to engineer a bacterial strain that was able to invade cancer cells in response to the hypoxic conditions of a tumor.29 Such systems may be adapted to provide targeted therapies for a number of human diseases through the production of therapeutic compounds only in the appropriate tissues or contexts. The bistable switch can also be generically applied to any strain for tight regulation of synthetic circuitry, for example using quorum sensing inputs to cause cell death,28 biofilm formation,27 or activation of protein synthesis in response to high population density.

Increasing complexity: genetic oscillators and circadian rhythms

Genetic networks of greater complexity can be created through the incorporation of additional genetic modules into simple synthetic networks.31 Even with only a few components, small changes can give rise to entirely new emergent behaviors; adding a third repressor to the bacterial toggle switch, thereby forming a mutually inhibitory ring gives rise to a simple oscillator, termed the “repressilator”. As with the toggle switch, synthetic oscillators make it possible to study natural oscillatory networks in a simplified context, as well as providing useful behaviors for further engineered systems. Natural oscillators such as circadian rhythms or heartbeats are controlled by many proteins and regulatory feedback loops, a large number of which have not been characterized.33 Even engineered transgene expression tied directly to known members of the circadian rhythm pathway were not able to produce robust oscillations, indicating that our knowledge of cellular clocks is at best incomplete.34 The work towards creation of synthetic oscillators has been instrumental not only to improving our understanding of biological clocks, but has also allowed for progress in the development of increasingly complex synthetic networks. Oscillatory control of transcription dynamics in synthetic gene circuits could be used in metabolic engineering, for the temporal regulation of pathway activity, or in biomedical applications where timing is important, such as drug delivery.

While the repressilator did function as expected over short time-scales, the oscillations became rapidly desynchronized across different cells in the population and were not maintained after several cell divisions, indicating that more complex regulation is required for the design of an oscillating circuit. More recent synthetic oscillators have improved mathematical modeling of circuit parameters and implementation in such a way as to closer mimic biological clocks in a variety of systems. Interestingly, many different topological arrangements of simple feedback loops can lead to robust oscillations. A synthetic oscillator based on coupled positive and negative feedback loops will maintain oscillations robustly in single cells over multiple cell divisions.35 This topology mimics the transcriptional network that is thought to control circadian rhythm in the mammalian brain.36 Mathematical modeling of this circuit was able to define relevant system parameters, indicating that a time delay in the negative feedback loop was critical for oscillations to occur, and that the rate of oscillations could be tuned through the action of the positive feedback loop.37 A similar system has since been implemented in mammalian cells for robust, tunable oscillatory expression of an arbitrary transgene, improving
our understanding of mammalian transcription dynamics with potential application to gene therapy.\textsuperscript{38}

As with all synthetic devices, these proof-of-principle experiments show great potential for novel application when arbitrary promoters and effectors are replaced with components of natural biological networks. Synthetic oscillators can be interfaced with native cellular machinery for oscillatory behavior that more closely mimics natural biological clocks and to produce biologically relevant output behavior. Connecting synthetic transcriptional oscillators with already pulsing biological networks can amplify oscillatory behavior,\textsuperscript{37} while oscillators linked to cellular metabolism through glycolytic flux will emulate the circadian rhythm’s control of metabolic activity.\textsuperscript{39} Integration of cell–cell communication machinery has been a standard practice for coordinating behavior between cells in many synthetic biology constructs, and has been shown to synchronize oscillations in populations of bacterial cells.\textsuperscript{40–42}

Electronics, evolution, and synthetic transcription circuits of the future

In the analogy to electrical engineering, the synthetic circuits described above are all transistors—electronic devices that can amplify or switch signals. More recent work has focused on creating other circuit components with analogy to diodes, which act as signal valves, allowing information to flow in only one direction, capacitors, which act as a buffer sustaining the circuit after removal of the signal, and resistors, which block the flow of the signal at a given rate. Different combinations of these components allow for the diversity of signal processing in electronic circuits, and are beginning to be explored in synthetic biological circuits. A genetic “diode,” “capacitor,” “resistor,” and “transistor” were connected to create a time-delay circuit in mammalian cells, a common network motif in biological systems.\textsuperscript{43} Instead of the signal molecule immediately binding the transistor and effecting the output, the signal is first bound by molecules that keep the signal inside the cell membrane (the diode), maintaining a pool of signal molecule in the cell even after washing (the capacitor), which then can either undergo proteolysis (the resistor), or bind to the transcription activator that binds to the promoter that produces the output (the transistor). The behavior of the circuit can be further modulated by addition of other chemicals that can interact with any of the components, creating a network that can process signals from multiple sources to give a coherent response.

Often when designing such complex systems, the ability of natural systems to evolve away from the engineered behavior is detrimental to experimental progress.\textsuperscript{44} Indeed, with the analogy of biological pathways as electronic circuits, one defines designed systems in opposition to evolved systems. However, taking advantage of evolution for engineering biological systems has played an important role in many synthetic biology devices and will continue to be useful as synthetic biology grows. In an early example of using directed evolution to engineer a cellular circuit, non-functioning synthetic networks were put through mutagenesis and selection in order to evolve the appropriate kinetic parameters for the desired function, bypassing the need for intensive quantitative characterization of the circuit components.\textsuperscript{45}

Furthermore, this directed evolution approach has great potential for developing new synthetic networks as well as improving our understanding of how mutations affect biological networks.\textsuperscript{46,47}

The modularity and evolvability of natural systems has also been used to discover novel topological arrangements of transcriptional regulators that perform a desired function through selection of randomized network connectivity \textit{in silico}\textsuperscript{48} and \textit{in vivo}.\textsuperscript{49} By building a combinatorial library of gene circuits with different connectivities from a handful of well-characterized promoters and repressors, Guet \textit{et al.} were able to screen for novel network topologies that function as Boolean logic gates, able to integrate two inputs into a single output. This type of experiment has consequences for future design of genetic circuits and for our understanding of how such circuits evolved naturally.\textsuperscript{50–52}

**Synthetic translational regulation and RNA devices**

RNA has in recent years risen from the position of middleman between DNA and protein to the likely origin of all biological chemistry. The discovery of ribozymes, riboswitches, and microRNAs has revolutionized the way we think about genetic regulation and cellular function, with cellular RNAs presenting a wide range of regulatory behaviors that can act as an interface between nucleic acids, proteins, and small molecules. Because of their ability to form complex secondary structures, RNA devices are highly modular, containing separable domains that can bind DNA sequence, proteins, and/or small molecules, as well as domains that can perform catalytic activities. All of these regulatory modules can be used to create novel synthetic RNA molecules for tight control over gene expression.\textsuperscript{53} RNA synthetic biology has tremendous potential as a tool to probe biological phenomena, both by mimicking natural gene regulation and as assay tools for basic science.\textsuperscript{54} RNA-based regulation modules can be easily integrated into synthetic devices as intergenic regions of designed DNA sequences, allowing for powerful control over synthetic circuits.

**Engineering intergenic regions**

Gene expression is controlled in large part by intergenic sequence modules. Introns not only affect how mRNA is alternatively spliced,\textsuperscript{55} they can affect the timing of gene expression,\textsuperscript{56} and contain miRNA sequences that can affect the translation of countless other genes.\textsuperscript{57} This level of control was discovered a relatively short time ago and new links are continually being added to our picture of the global miRNA network. In synthetic biology, miRNAs have been encoded into intronic sequences as repressors that can tune the response of a transcriptional switch in mammalian cells.\textsuperscript{58} By binding to and causing the degradation of the mRNA transcript encoding the output signal, these miRNAs effectively silence any leaky expression from the synthetic gene, allowing for tightly controlled, reversible, and tunable expression of toxic transgenes in mammalian cells and live animals. Replacing input and output modules with genes of
Moreover, this synthetic RNA system is useful to the study particularly those where rapid expression is important. Any number of synthetic transgene expression systems, including antisense-mediated riboregulation of genes in prokaryotes, can be used to study the effects of gradual expression changes of a transgene or of otherwise lethal gene knockouts during different time points in embryonic development.

Other intergenic modules that have been used to control gene expression in synthetic networks include ribosome binding sites (RBS), which can be tuned to control the strength of translation, or intergenic regions that can form hairpins and destabilize the mRNA transcript. This type of transcriptional regulation can be used to fine-tune expression of each member of a synthetic metabolic pathway, optimizing output while minimizing the genetic load on the host cell.

Another level of synthetic RNA control can be achieved through the use of a simple antisense sequence coded into the 5' UTR of the synthetic transgene. This sequence forms a hairpin in the mRNA sequence, binding to the RBS and repressing translation in cis (Fig. 3A). This cis-repressing RNA (crRNA) prevents translation of the transgene until the hairpin is destabilized by a second, complementary non-coding RNA expressed in trans off of a second promoter. This system allows for rapid, tunable expression of the cis-repressed transgene within minutes of trans-activating RNA (taRNA) expression. As a modular synthetic system it can be applied to any number of synthetic transgene expression systems, particularly those where rapid expression is important. Moreover, this synthetic RNA system is useful to the study of antisense-mediated riboregulation of genes in prokaryotes, such as the hok/sok system that maintains plasmid R1 in a population by killing daughter cells that do not contain the plasmid.52

Designed ribozymes and RNA logic devices

Modular ligand-binding trans-acting RNA devices, termed antiswitches, control gene translation in a similar manner in eukaryotic cells (Fig. 3B).63 Antiswitches are modular RNA sequences that change conformation upon binding of a small molecule ligand, affecting translation of mRNA with complementary sequence in trans. Antiswitches are made up of a DNA binding site, a small molecule inducer binding site, and a linker domain bringing the two together. In the absence of inducer, the DNA binding domain based-pairs to the RBS, blocking translation. With the addition of the inducer molecule, the secondary structure of the antiswitch changes such that it can no longer bind to the RBS, allowing for expression of the transgene. A similar scheme can also be used to generate a repressor, where addition of a different small molecule will allow for binding, preventing translation initiation.

Antiswitches are modeled after natural riboswitches, cis-acting secondary structures that bind to cellular metabolites and undergo structural changes in order to regulate gene expression. Riboswitches respond to a wide range of biological molecules, including adenine, glucosamine-6-phosphate, coenzyme B12, thiamine pyrophosphate, flavin mononucleotide, S-adenosylmethionine, and lysine.64 RNA aptamers, small domains that bind to chemicals, can be evolved to interact with any number of small molecules with SELEX, Systematic Evolution of Ligands by EXponential enrichment.65 Synthetic RNA devices incorporating such aptamers can sense a wide array of target molecules, including peptides, ions, dyes, and antibiotics.66 These aptamers can then be attached to any natural or synthetic RNA device, including trans-acting antiswitches and cis-acting ribozymes. Self-splicing ribozymes encoded into the mRNA sequence of a target gene will lead to spontaneous cleavage of the transcript. Addition of inducer will stabilize an alternate secondary structure, allowing for translation of the mRNA transcript. Synthetic self-splicing ribozymes have been engineered to control gene expression in mammalian cells, with potential application for regulated delivery of protein-based therapeutics.67

Increasingly complex behaviors are achieved in natural systems through different combinations of RNA control modules. For example, two tandem riboswitches or single riboswitches with multiple metabolite binding domains exhibit cooperative control over natural gene expression. Based on these natural systems, combinatorial design of synthetic ribozymes leads to more complex circuit behavior, with devices that can activate or repress genes of interest with the addition of different combinations of chemical inducers.69 A synthetic gene with two self-splicing ribozymes in the 3' UTR can perform very different signal integration behavior depending on the combination of aptamer and catalytic domains. If both aptamer domains respond to the same inducer molecule, the sensitivity of the system is decreased, with less transgene expression per unit inducer. The system acts as a logic gate when each ribozyme responds to a different inducer. With effector domains that activate translation in
response to inducer this configuration gives an AND gate, where both inducers are required for expression. Conversely, two ribozymes that splice in the presence of inducer create a NOR gate, where the gene is translated only when neither inducing molecule is present. Similar behavior can also be engineered into a single composite ribozyme with a multiple chemical binding sites. These units can be further recombined in future synthetic devices for more complex control over circuit function.

Signal transduction

Nucleic acids can perform a wide array of important circuit behaviors and have proven to be valuable synthetic biology parts. In cells, signal transduction proteins provide a further layer of information processing, integrating signals from multiple sources inside and outside the cell into coordinated cellular activity. Signaling proteins are highly modular; protein–protein interaction subunits such as SH2, SH3, or PDZ domains are functionally separable from activity elements, such as kinase or phosphatase domains. Pathways of highly regulated protein interaction events create many common regulatory motifs, including feedback loops or bi-fans—where two inputs cross-regulate two outputs. Different domains of signal transduction proteins are often encoded by separable parts of the gene, allowing for these domains to be easily integrated and recombined into synthetic gene circuits. Current examples of synthetic signal transduction pathways show how new cellular behaviors can arise through recombination of existing signaling domains. These designed systems likely reflect how the complexity of the signal transduction network evolved—through recombination, duplication, and drift between simple interaction and activity elements.

Signal transduction is a vibrant area of cell biology research, and further advances in our understanding of how cellular information processing proteins work together will improve the ability of synthetic biology to engineer cellular behavior and vice versa. The re-wiring of well-understood signaling pathways can show us what we understand well and where more research is needed. This has implications for basic science and for medical research, as signal transduction plays a major role in the progression of many cancers. For example, signaling proteins have been recombined to create protein based medicines that specifically target cancer cells. Pro-apoptotic protein therapies have many negative off-target effects. Mutating the apoptotic signal protein so that it can only weakly bind cells and physically linking it to a protein that can tightly bind receptors found only on cancer cells creates a chimeric protein that will specifically target cancer cells and kill them. Because of the modularity inherent in this system, chimeric protein therapies can be made to target any cell that has a specific surface marker and affect any cellular behaviors that have surface receptors. Signaling pathways can also be re-wired inside the cell through genetic engineering to create novel cellular behavior and better understand how signal transduction evolved.

In bacteria, most signal transduction is performed by two-component systems made up of a histidine kinase and its cognate response regulator (Fig. 4A). Histidine kinases can sense a wide array of external signals, including temperature, osmotic pressure, metabolite concentration, and chemotaxis molecules. Upon ligand binding, the histidine kinase autophosphorylates and transfers the phosphate group to a response regulator that activates a cellular response. Response regulators can be DNA binding transcription factors, bind RNA or protein, or perform enzymatic activities. Tapping into the amazing diversity of bacterial two-component system modules have made it possible to engineer E. coli to respond to light through fusion of a cyanobacterial photoreceptor to the histidine kinase EnvZ. EnvZ has also been engineered to recognize different response regulators, showing that it is possible to connect an arbitrary output behavior to a new input signal. Like the transcriptional switch, bacterial two-component systems can be used to activate useful cellular responses to any signal of interest in order to create biosensors, study biological phenomena, or better regulate synthetic pathways with environmental signals.
More complex in vitro signal integration behavior can also be developed through recombination of existing eukaryotic signal transduction domains. Synthetic autoinhibition of signaling proteins can be achieved through the linking of an activity domain with self-interacting elements that close over the active site. Addition of exogenous ligand competes with the interacting domains, allowing for activity (Fig. 4B). More complex systems can be built through the addition of more such interacting domains. Addition of more domains of the same type leads to ultrasensitive behavior, while having two ligand-binding domains from different signaling proteins yields an AND logic gate. Autoinhibition is a common characteristic of natural signaling networks including guanine nucleotide exchange factors (GEFs), which control a wide range of biological activities, including regulation of the actin cytoskeleton. Recombining the autoinhibitory module and the activity module from two different GEFs causes mammalian cells to undergo morphological changes in response to non-native inputs in vivo. These synthetic signaling proteins demonstrate how novel signal transduction behavior could have evolved through the evolutionary recombination of only a few interacting domains.

Co-evolution of these interacting pairs of signaling proteins limits cross-talk between different pathways in cells. While this is thought to be the primary method of pathway isolation in prokaryotes, much of the regulation that prevents cross-talk between the similar interaction domains of signal transduction proteins in eukaryotes happens in a context-dependent way. Signal transduction pathways with similar domains are functionally isolated either through temporal regulation, organelle or tissue specificity, or through scaffold proteins that recruit interaction partners. In synthetic biology, recombined scaffold modules can be used to connect disparate cellular pathways. For example, parts of scaffold proteins required for the function of two mitogen-activated protein kinase (MAPK) pathways in S. cerevisiae, osmotic response and mating-type switching, were recombined so that the opposite output cascade could be activated by the opposite inputs (Fig. 4C). Studies such as these will be able to show us how scaffold proteins and connectivity between signal transduction proteins affect signaling responses, give insight into the evolution of complex signaling processes, and provide tools for engineering mammalian cellular pathways for therapeutic purposes.

Conclusion

Through an engineering based approach to understanding and using biological molecules, synthetic biology has been able to design and implement many potentially useful biological circuits. Synthetic biology aims to build biological systems out of abstracted, modular parts, wrapping a “black box” around increasingly complex natural or engineered networks that can be chosen and recombin in a “chassis” cell that will use the pathways towards a desired function. This approach has been successful in the design of switches, oscillators, and sensors out of transcription, translation, or signal transduction based modules. However, while there has been great success in creating simple networks out of modular DNA or RNA parts in recent decades, the higher-order recombination of standardized interacting pathway modules has remained elusive.

While the abstract jump from simple circuits to complex machines is clearly defined and well understood in computer science and electrical engineering, in biology the transition from understood biological pathways to “life” is still unclear. What makes self-organized and evolving biomolecules lead to the incredible complexity of biological phenomena is the last frontier of vitalism, a belief that living things possess a magical spark that brings them to life. This belief is part of the argument that living things are “irreducibly complex” that is explicit in the religious arguments against a rational understanding of biological phenomena, and implicit in many critiques of synthetic biology. Just as synthetic chemistry eliminated vitalism from the study of organic molecules, so too must synthetic biology shatter the notion that biological systems are fundamentally special in some way that prevents them from being rationally engineered.

While synthetic biology has shown that it is possible to engineer simple biological networks with predictable function, much of the promise of synthetic biology remains in the future. Synthetic biology has the potential to revolutionize drug production and delivery, chemical sensors and bioremediation, bio-energy, tissue engineering and gene therapy, and importantly, the study of biology itself. The tools and techniques of synthetic biology—mathematical modeling, standardization, modular cloning, and vector design—combined with inexpensive gene synthesis and sequencing will greatly improve our ability to study biological systems and make new discoveries easy to incorporate into other synthetic networks.

Moreover, thinking of biology in terms of interchangeable modules gives new insight into the nature of biological systems as a whole, as well as a new way to approach biology experimentally. Just as synthetic biology relies on systems biology for a global and quantitative understanding of the underlying cellular networks, so too does systems biology need synthetic biology experiments to test hypotheses and develop understanding of biological network robustness, noise processing, and evolution. The ability to engineer biology has shown us how these complex cellular information processing systems can evolve from interactions between simple parts. Indeed, synthetic biology has shown how biological processes can act as engineered computation machines, with examples of biological molecules used as computers and logic gates. A deeper understanding of how biological networks adapt and evolve will likewise have consequences for the development of new computer science methods. Through synthetic biology, biology and engineering will continue to productively affect one another in the years to come, with consequences for medicine, biological research, and technology.

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