Leading Edge
Review
Cell

Integrating Biological Redesign: Where Synthetic Biology Came From and Where It Needs to Go

Jeffrey C. Way,[1] James J. Collins,[1,3] Jay D. Keasling,[4,5,6,7] and Pamela A. Silver[1,2,7,*]
[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
[2]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
[3]Howard Hughes Medical Institute, Department of Biomedical Engineering and Center of Synthetic Biology, Boston University, Boston, MA 02115, USA
[4]Department of Chemical and Biomolecular Engineering, Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720, USA
[5]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[6]Joint Bioenergy Institute, Emeryville, CA 94608, USA
[7]Synthetic Biology Engineering Research Center (SynBERC), University of California, Berkeley, Berkeley, CA 94720, USA
*Correspondence: pamela_silver@hms.harvard.edu
http://dx.doi.org/10.1016/j.cell.2014.02.039

Synthetic biology seeks to extend approaches from engineering and computation to redesign of biology, with goals such as generating new chemicals, improving human health, and addressing environmental issues. Early on, several guiding principles of synthetic biology were articulated, including design according to specification, separation of design from fabrication, use of standardized biological parts and organisms, and abstraction. We review the utility of these principles over the past decade in light of the field's accomplishments in building complex systems based on microbial transcription and metabolism and describe the progress in mammalian cell engineering.

Introduction

Synthetic biology is a young discipline with the declared goal of rationally engineering biological systems through approaches similar to those used by engineers to build bridges and send people to the moon. This field has rapidly developed over the past ~15 years from its initial conceptualization by a few academics and government program managers into a sizeable field whose meetings attract large numbers of participants (http://sb6.biobricks.org/).

The need for synthetic biology is certainly real. The moon-shot challenges for the present generation of scientists working in the field address a spectrum of urgent, real-world issues. It would be ideal to engineer biofuel production from photosynthetic systems to address the problems of global warming and energy self-sufficiency (Kung et al., 2012). Complex diseases such as cancer and autoimmune disorders may simply never yield to simple target/inhibitor types of drug approaches and may require complex engineered proteins or cells (Bagshawe, 2009; Chester et al., 2002; Porter et al., 2011). Food shortages may become an issue as the world's population continues to increase exponentially, while production increases from the green revolution reach their limit (Fischer et al., 2009; Bomford, 2009). In its move toward such goals, synthetic biology also made an impact on the thinking about biological systems, redefining organisms such as microbes previously appreciated for their basic biology or pathogenic properties, in terms of their value for biological redesign.

A framework for synthetic biology was proposed soon after its inception as a discipline, and the developments in the last decade provide an opportunity to look both backward and to the future. This Review addresses where synthetic biology needs to go to have a maximal impact and places it into the context of existing disciplines.

Before Synthetic Biology: Recombinant DNA

The significance of the recombinant DNA revolution that started in the mid-1970s cannot be overstated. It has led to new drugs, metabolically engineered microbes that make diverse nutraceuticals and commodity biochemicals (e.g., most of the vitamins in vitamin pills), modern genotyping and DNA testing, and genetic techniques that have transformed the way scientists analyze biological systems. In the pharmaceutical industry alone, recombinant proteins are used as monoclonal antibodies and other protein drugs, as well as drug targets used in high-throughput screening. However, if we look at these accomplishments, it is important to note that recombinant DNA products are limited in their complexity—single proteins, small molecules based on screening against single proteins, and metabolically engineered microbes that have been developed through extensive trial and error. We know how to make individual proteins, but it goes beyond current practice to create new multicomponent systems that mirror natural biology in their complexity. The promise of synthetic biology is to do just that. In particular, the promise of synthetic biology is to construct organisms with genuinely novel features that represent a jump from what already exists. Such constructs can be thought of as working systems that are separated from what currently exists by

nonworking intermediates. Evolution generally requires working intermediates. Because the systems that synthetic biologists want to construct are complex and involve numerous variables with values that must be in a narrow range for the system to work (e.g., expression levels, binding constants, Hill coefficients, and geometry of interactions), it is often not feasible to arrive at solutions simply by intuition, guesswork, or incremental modification of an existing system. To think about an analogous problem, consider the actual moon shot. This audacious program was not achieved through incremental changes from existing systems, for example, by sending millions of randomly varied test rockets to the moon, seeing which one makes it, and then copying that design. The parallel to biology is that evolution can only get you so far over short time scales. Instead, as with the moon shot, extensive design and calculation are required to make big leaps to new biomolecular creations.

Formulating the Synthetic Biology Framework

Approaches to synthetic biology were most extensively explored a decade ago, during a Defense Advanced Research Projects Agency (DARPA)-sponsored study (Endy et al., 2003) that brought together biologists, bioengineers, computer scientists, and hardware developers and resulted in a recommendation that the field of synthetic biology should learn from the earlier developments in the microchip industry. This industry took dramatic steps forward in the late 1970s by setting up specific standards for chips, making them modular and easy to connect, and through the formation of MOSIS, a DARPA-sponsored program that supported the design and testing of chips (http://www.mosis.com/). These measures rapidly led to the formation of the integrated circuit industry, accompanied by several social developments such as the separation of programming from chip manufacturing. In synthetic biology, the corresponding goal is to separate DNA synthesis from construct design and to develop a class of bioengineers who would approach biology with the mindset of computer programmers and chip designers. The study ultimately led to the formulation of a synthetic biology framework that, although not universally accepted, has defined important elements in the thinking about how synthetic biology could be practiced.

Design to Meet Specifications Set in Advance

This element posited that, as in other engineering fields, the specifications for a design should be quantitative and relate to the ultimate use of a genetic element. As an analogy, one can think of a design of physically engineered elements—"I need a bridge that will support 50 tons of vehicles for 100 years" or "I need a circuit board that will perform calculation X at a speed of 10^6 calculations per second and operate between 0° C and 50° C." Corresponding requirements for a biological system might be "I need a genetic memory element that will maintain a stable state with a spontaneous switching rate of $<10^{-4}$ per cell division in Gram-negative γ-proteobacteria growing at a rate of 0.3 to 3 doublings/hour." Such specifications may ultimately become routine but currently are employed by only few members of the synthetic biology community.

Separation of Design from Fabrication

In its most extreme form, this recommendation would correspond to a separation of synthetic biology projects into software and hardware efforts, with the software aspect focused on design of constructs and the hardware aspect focused on making DNA, RNA, or proteins. The ideal outcome of this element would be faster system testing.

Use of Biological "Parts"

This principle relates to the issue of modularity in biology and is intuitively obvious to most molecular biologists. The gene has long been the central modular unit of biology, but with the advent of molecular biology, genes, RNAs, and proteins have been further dissected into functional modules such as promoters and protein domains. An open question is whether biology is genuinely modular in an engineering sense or whether modularity is only a human construct that helps us understand biology. To clarify, it may be useful to distinguish between "concept modularity" and "engineering modularity." For example, to understand the process of translation, it is useful to think of a ribosome binding site and a coding sequence as separate modules. However, from an engineering point of view, these elements are not necessarily distinct modules because they can interact through mRNA secondary structure, with levels of translation resulting from a nonlinear combination of the two elements (Goodman et al., 2013; Gardner and Hawkins, 2013).

Abstraction of Biological Elements

Abstraction is an important aspect of how humans view and engineer the world. In the computer industry, physical structures made of silicon, germanium, etc., are abstracted as "memory," and a programmer can ignore what comprises the memory. Biological engineers routinely perform such abstractions, representing proteins as circles, nucleic acids as lines, and collections of metabolites as vectors in metabolic flux models. Development of software tools for biology greatly benefits from such abstraction; for example, the caDNAno program abstracts DNA structure to allow design of DNA origami nanostructures (Douglas et al., 2009). One potential issue with this recommendation is whether such abstractions interfere with biological engineering by depicting as black boxes elements that could and should be further characterized and engineered.

Well-Defined "Chassis" Organisms

Another parallel to engineering is that a completely defined cell would be ideal to serve as a chassis into which engineered biological systems would be integrated. This approach could potentially eliminate the vagaries associated with the unknown features of a natural cell. The so-called "minimal cell" has been sought through both top-down (removing as many genes as possible; Hutchison et al., 1999; Sharma et al., 2007) and bottom-up approaches (resynthesis of new organisms, for example, with alternative genetic codes, by combining the genome synthesis approach of Gibson et al. [2010] with the recoding strategy of Lajoie et al. [2013]). A number of methods for genome editing have surfaced in the last 10 years, making major changes in genomic DNA faster and more predictable (Wang et al., 2009; Gaj et al., 2013).

It has been 10 years since these ideas were put forth, and it is thus worthwhile examining the success of the framework. Not surprisingly, and as described in the following sections, some elements have been more useful than others.

Employing Design Specifications: A Cultural Divide

Meeting design specifications in synthetic biology turned out to be a major challenge because it often underscores the real difficulty of a given biological problem, especially for ambitious projects such as cell-based disease treatments. However, design specifications are routinely used in metabolic engineering, particularly with regard to production efficiency versus production cost. In our experience, the notion of design specifications highlights a cultural divide between engineers and biologists. Engineers learn the concept of design specifications in college, whereas biologists trained in discovery research focus on uncovering new things about nature without preconceptions. Starting with a fixed idea of what is to be achieved, which is at the core of synthetic biology, may inhibit discovery research while being essential to the creation of new objects, whether physical or biological. To enable synthetic biology, explicit training in design-to-specification will be needed at an early stage in the careers of biologists.

Separating Design and Fabrication: Hardware Outpaces Software

The implementation of this element has been decidedly mixed. On the hardware side, DNA synthesis is becoming cheaper and cheaper. Following the microchip analogy, Carlson (2009) described this phenomenon as exceeding ''Moore's Law,'' which, describing the rapid rise of integrated circuits, postulated that increases in computer power double approximately every 2 years. On the other hand, although there is an ongoing evolution toward outsourcing DNA constructions, graduate students and postdocs still spend much of their time making plasmids. The real transition will come when DNA synthesis is so cheap that a graduate student no longer needs to ask an advisor for permission to build a new microbial genome or an entire plant chromosome, for example. The design aspect has been more challenging. The Platonic ideal would be ordering a set of DNA constructs and placing them in an organism where they would work with minimal tweaking and optimization. What is necessary for such a successful design? There has been some effort in the development of design tools that address very specific purposes, such as flux-balance programs for metabolism, translation calculators for optimization of ribosome binding sites (Salis, 2011), caDNAno for designing oligonucleotide-based origami structures (Douglas et al., 2009), protein structure visualization programs, RosettaDesign for engineering protein domains (Tinberg et al., 2013), as well as tools for engineering multidomain proteins (Robinson-Mosher et al., 2013), and numerous other pieces of software (Kahl and Endy, 2013). Many applications in synthetic biology would require most or all of these tools—in the course of evolution, nature changes all of these features at the same time. At present, the majority of these computer-based design tools allow ruling out designs with fatal flaws, but extensive trial and error is often still required to obtain a working prototype. Moreover, the use of these tools requires both a familiarity with the tools themselves and a decent understanding of the biological process being modulated; such a breadth of understanding often goes beyond current educational practice.

Biological Modules: Learning from Nature

The use of biological modules has been championed by the Registry of Biological Parts, SynBERC, and others (Shetty et al., 2008; Kosuri et al., 2013; Kobayashi et al., 2004; Mutalik et al., 2013a, 2013b). The underlying challenge is to collect, characterize, and catalog DNA elements with potential utility. A registry serves as a collection point for information, as well as for DNA elements themselves, although the latter function may become irrelevant as DNA synthesis becomes sufficiently cheap to allow creation of such ''parts'' from scratch, rather than obtaining them from a registry and assembling them. A registry would then remain a storage point for information and standards. Another element of the original concept was devising a universal connecting method to facilitate construction of progressively more complex parts. The original BioBricks standard (Knight, 2003) involves a restriction enzyme-based cloning strategy in which ligation eliminates the medial cut-sites so that the resulting DNA becomes a larger part. This approach is becoming less relevant in light of new technology; specifically, the assembly method of Gibson et al. (2009) and other related strategies (Kosuri et al., 2010; Dharmadi et al., 2013), which are restriction-site-independent methods for assembling DNA and which may someday replace most restriction enzyme-based cloning. The design of artificial modules has been often inspired by natural genetic modules, such as the lactose operon and pathogenicity islands in bacteria. It is, however, important to point out that the modularity of these units is a consequence of natural selection—they are thought to be transferred between different types of bacteria by transduction and conjugation, becoming self-sufficient through natural selection, and to able to function in diverse bacteria that may vary in other ways, such as their central metabolism or ribosomal RNAs that recognize Shine-Dalgarno sequences. Other biological processes, such as cell membrane construction or chromosomal replication, may be nonmodular, but this will be less apparent from the genetic organization. Protein domains constitute another example of genetic modules. There are numerous examples of large proteins that have presumably arisen from fusion of smaller units, and it appears that such fusions are a common occurrence during evolution. For example, the SH2, SH3, protein kinase, fibronectin Type III, immunoglobulin, and WD40 domains are used in signaling proteins in a wide variety of configurations and contexts, arising by exon shuffling and similar mechanisms (Gilbert, 1978; Bork et al., 1997). It seems likely that, after initial formation, new fusion proteins may be suboptimal and may further evolve by point mutations and changes in interdomain segments to adjust to their biological context. The point for synthetic biologists is that modularity can be a useful place to start, but additional conceptual tools are needed to improve protein constructs before and after modules are assembled (Cironi et al., 2008; Robinson-Mosher et al., 2013).

Abstraction: A Double-Edged Sword

In the context of biological engineering, it is still an open question whether abstraction is a useful tool or a necessary evil.

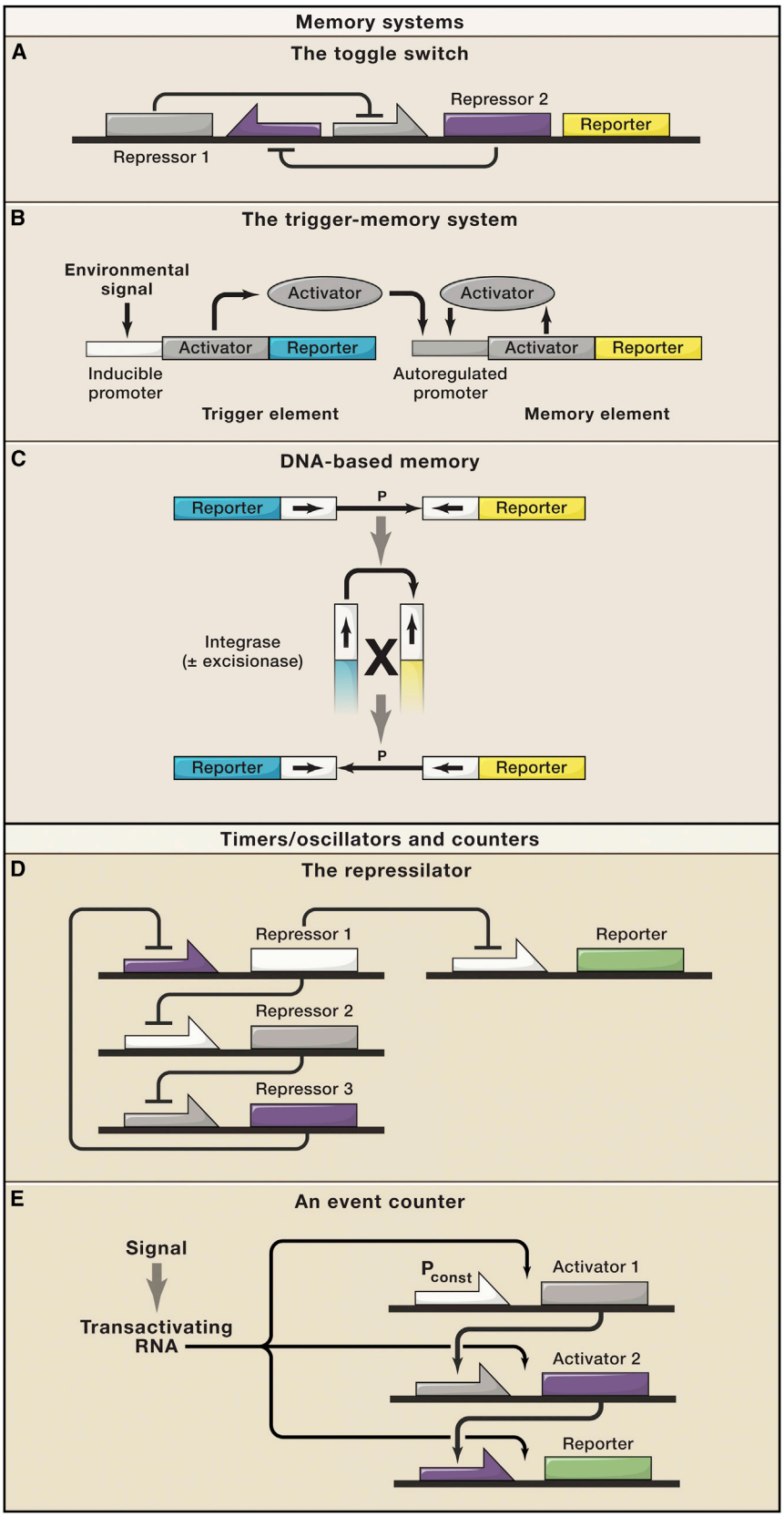

Figure 1. Synthetic Biological Devices Based on Gene Expression

(A) The toggle switch, in which two repressors turn each other off, leading to a bistable transcriptional state (Gardner et al., 2000).

(B) The trigger-memory system, in which an environmental stimulus induces an activator (the trigger) that then turns on its own synthesis in a second "memory" element, leading to a permanent "ON" state (Ajo-Franklin et al., 2007; Burrill and Silver, 2011; Burrill et al., 2012).

(C) A DNA-based memory element, in which activation of an integrase or integrase + excisionase leads to alternating DNA states (Bonnet et al., 2012, 2013; Siuti et al., 2013).

(D) The repressilator, in which three repressors sequentially turn each other off, such that the state of the system oscillates in time (Elowitz and Leibler, 2000).

(E) An event counter, in which a signal turns on a positive regulator of translation and acts in combination with sequentially expressed transcriptional activators to count pulses of a signal (Friedland et al., 2009).

Computer hardware can be abstracted to the point where a circuit can be represented simply in terms of its inputs and outputs. Our ability to perform such an abstraction results from our ability to design computer hardware around human cognitive capabilities. Biological systems, in contrast, have been created by evolution and are not necessarily abstractable in ways that the human brain has evolved to handle. For example, the massively parallel function of metabolism, Brownian motion, allosteric changes in protein conformation, and movement in a low Reynolds number regime are examples of processes that are difficult to abstract and intuit, which may lead to poor design in biological engineering. The challenges posed by abstraction can be illustrated by two examples from the synthetic biology field.

The "Bag of Genes" Abstraction of a Cell

Synthetic biologists have spent a great deal of time developing artificial transcriptional systems (Figure 1). In fact, one of us (J.J.C.) recently edited an issue of the journal *Chaos* focused on synthetic biology, with most articles describing transcriptional systems (Albert et al., 2013). Transcriptional networks are attractive because their behavior can be modeled as a "bag of genes"—analogous to the biochemists' "bag of enzymes"—through various reasonable abstractions and because the key biochemical values have been determined for a number of bacterial systems (Huynen and Bork, 1998; Mathews, 1993; Arkin et al., 1998). For example, the bacteriophage λ system has been a favorite of mathematical modelers, who have rationalized the lysis/lysogeny decision and the decision to exit or enter the lysogenic state (Ackers et al., 1982; Arkin et al., 1998). The modeling is possible because many of the quantitative parameters of these systems have been determined over the past 40 years. Based on this background, Gardner et al. (2000) and Elowitz and Leibler (2000) constructed simple transcriptional devices that behaved either as a toggle switch or an oscillator (Figures 1A and 1D). These early examples of synthetic-biological circuits suggested the possibility of designing artificial transcriptional circuits that could perform useful tasks. Since then, there has been much follow-up work on transcriptional circuits. Kobayashi et al. (2004) demonstrated that it is possible to link promoters to output molecules to construct new genetic devices and programmable cells, which was a natural extension of the use of constructs for reporters, protein expression, and protein relocalization that had been used since the mid-1970s (Casadaban, 1976; Backman and Ptashne, 1978; Silhavy et al., 1976). Use of RNA binding to an mRNA offers another regulatory mode that is easily programmed, building on base-pairing rules and energetic considerations that can be translated into simple design rules (Isaacs et al., 2004; Callura et al., 2010). Certain DNA binding proteins such as zinc-finger (ZF) proteins, and especially TALEs, have a roughly one-to-one correspondence between certain amino acids and the DNA that is recognized, allowing straightforward programming of transcription as well (Elrod-Erickson et al., 1998; Garg et al., 2012; Lienert et al., 2013; Bultmann et al., 2012; Khalil et al., 2012). These features make plausible the idea that biological systems could be programmed by simple rules to allow biological computation and have thus attracted a great deal of attention from the synthetic biology community. That being said, the focus on gene expression has its limitations. First, gene-expression-based circuits are relatively slow. Even in bacteria, transcription plus translation to yield an average-sized protein (340 aa at 17 aa/s translation; Young and Bremer, 1976) would take about 20 s, compared to the subnanosecond calculation speed in a typical personal computer. Biological systems do perform rapid computations, using neurons for example, but these fast mechanisms do not involve transcription or translation. Second, the inputs and outputs will likely be the more interesting things about a biological circuit. DNA-protein interactions may be somewhat programmable, but interactions with the chemical and physical environment are much more difficult to engineer. A holy grail of synthetic biology and protein engineering is to design macromolecules that can recognize an arbitrarily chosen ligand, with coupling to an arbitrary enzymatic activity. Artificial fusion proteins with allosteric properties have been constructed (Guntas et al., 2005; Meister and Joshi, 2013), but their function may depend on the particular geometry of the fusion partners, and it is difficult to know how general this approach may be. RNAs have been identified that bind to various ligands, and these have been further engineered to create sensors that regulate gene expression (Win et al., 2009). However, it would be ideal to be able to start with the large number of natural ligand-binding elements, most of which are protein based, rather than needing to start a project by isolating RNA aptamers that bind to a target of interest. This approach has led to biological computers of general programmability that can be built from transcriptional systems. These are slow and will never approach the speed of silicon-based computers. Their real value will likely be that biological computers can be embedded in the environment and "live off the land" while recording and calculating events of interest. Genetic memory devices such as the toggle switch and its offspring could be particularly useful in this regard (Gardner et al., 2000; Ajo-Franklin et al., 2007; Burrill and Silver, 2011; Burrill et al., 2012; Bonnet et al., 2012, 2013).

The "Bag of Enzymes" Abstraction of a Cell and Its Limitations

Metabolic engineering involves the genetic manipulation of microbes to overproduce compounds of commercial interest (Keasling, 2012; Keasling et al., 2012). Synthetic biology has the potential to significantly extend the reach of metabolic engineering through the use of fused and scaffolded enzymes to direct flux and enhance yields (Dueber et al., 2009; Agapakis et al., 2010; Delebecque et al., 2011; Whitaker and Dueber, 2011) and through the creation of novel pathways that can synthesize natural or nonnatural compounds (Moon et al., 2009; Steen et al., 2010). If properly harnessed, enzymatic chemistry could create thousands of diverse, novel molecules and materials. The main tools of metabolic engineering have been gene knockouts, addition of foreign pathways into high-producing hosts, and analysis of flux patterns. One programmable aspect of metabolic engineering derives from simple conservation of mass and has led to genome-scale flux-balance models of cellular metabolism (Feist and Palsson, 2008; Oberhardt et al., 2009). This approach is the essence of the "bag of enzymes" view of the cell—the cytoplasm is a vessel in which enzymes and substrates are evenly distributed throughout the cell. Recently, several groups have engineered channeling and the compartmentalization of metabolism with the goal of enhancing

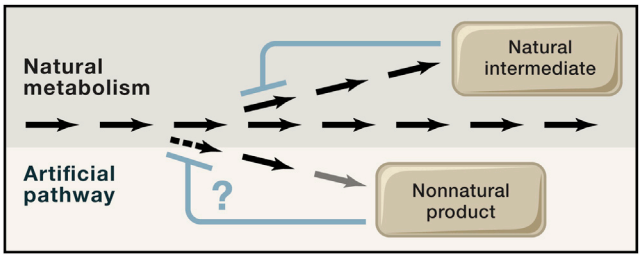

Figure 2. Control of Flux in a Cell Making a Nonnatural Product
In natural metabolism, almost every branch-point step is transcriptionally and/or allosterically regulated. One goal of synthetic biology is to make nonnatural products, which may be achieved by piecing together an artificial pathway of enzymes that may be from different sources or engineered to have new specificities. However, in such cases, there may be no mechanism for regulating the branch point at which flux is siphoned off from central metabolism. This problem is particularly acute if some carbon flux through central metabolism is needed to generate ATP or reducing equivalents. It would be ideal to engineer compound recognition into either a transcription factor or an allosteric site, but this is beyond our current protein engineering capability.

overall rates and limiting side reactions. Cells naturally use compartments to protect the contents from the cytoplasm, as well as the cytoplasm from the contents, to create a unique chemical environment and to concentrate chemical reactions (Mathews, 1993; Cheng et al., 2008). Dueber et al. (2009) placed three enzymes catalyzing mevalonate synthesis on a protein scaffold and observed an almost 80-fold synthesis enhancement. Delebecque et al. (2011) placed hydrogen-synthesizing proteins on an RNA scaffold and found a progressive increase of product synthesis as the dimensionality of the scaffold was increased from a zero-dimensional single RNA, a linear scaffold, to a 2D scaffold that gave an ~40-fold increase. These approaches draw on natural examples of substrate channeling and illustrate how moving beyond a simplistic conception of the cell as a "bag of enzymes" can be useful (Mathews, 1993). The synthesis of compounds that are not normally made by biological systems is an important new area at the intersection of synthetic biology and metabolic engineering. The goal is to use biological chemistry to make molecules that might otherwise be made using synthetic chemistry. For example, Atsumi et al. (2008) produced a set of related alcohols, some known and some new to biology, by mixing and matching enzymes to drive the production of various 2-keto acids, followed by decarboxylation with a nonspecific 2-keto acid decarboxylase and an alcohol dehydrogenase to reduce the resulting aldehyde. Similarly, Steen et al. (2010) described the synthesis of a variety of biofuel-type molecules and precursors, including C12- and C14-length fatty alcohols that are not normally produced biologically but whose production is engineered by coexpressing medium-chain fatty acyl thioesterases and nonspecific reductases from different organisms. Moon et al. (2009) created an artificial pathway for production of glucaric acid by combining enzymes from *E. coli*, yeast, and mammals. These examples illustrate how combining enzymes from diverse sources into a single cell can yield novel chemicals that are normally made only by synthetic organic chemistry or are not made at all. An important next step will likely be to combine this approach with protein engineering to further diversify the space of biochemicals. One future challenge may be that there are no regulatory proteins that can sense such novel end products, so engineering feedback inhibition may be difficult (Figure 2), underscoring the need for the integration of protein engineering into synthetic-biological systems.

Chassis Organisms: Moving beyond Microbes

An ideal chassis organism would be one that is so well understood that, when engineered DNA is added, there are no surprise interactions between the added material and host functions. One scenario would be a "minimal" organism—an organism built from the bottom up in which each gene and protein is present for a reason. Some effort has gone into generating such organisms by systematic gene deletion in an organism with an already small genome, for example (Glass et al., 2006). However, for microbial engineering, it is not clear whether unexpected or fortuitous interactions between added and endogenous factors might be a problem (though minimal organisms may help us test it). This is in sharp contrast to mammalian cell engineering in which one major problem is that unpredictable long-range interactions along the DNA in mammalian chromosomes occur all the time.

Much of the preceding discussion applies to microbial synthetic biology but does not carry over to mammalian cells. In particular, the bedrock technologies of synthetic biology—complex but reproducible genetic engineering and quantitative control—are not currently as feasible in mammalian cell engineering. A mammalian chassis for synthetic-biological circuits would have great utility.

Mammalian cell engineering, which could have a profound impact on treatment of disease, can boast of several major accomplishments. Production of monoclonal antibodies and other proteins has had a profound impact on the pharmaceutical industry, with protein drugs comprising almost 50% of total drug revenues and a higher efficiency of approval than chemical drugs (Rader, 2012). A few of these proteins are made in bacteria or yeast, but most are produced from mammalian cells because these cells mediate natural mammalian glycosylation, are better at catalyzing the folding of many proteins, and do not produce inflammatory contaminants (e.g., bacterial cell wall material) that must be completely removed before injection. However, the engineering of mammalian cells often involves laborious, inefficient methods. Cells are transfected with expression constructs that are typically integrated randomly into the genome (Murnane et al., 1990; Merrihew et al., 1996), although new targeted integration technologies may change this (Gaj et al., 2013). Expression levels vary depending on the integration site, as well as on epigenetically controlled chromatin structure around the inserted transgene. It is possible to identify cell clones that constitutively express one or two proteins (e.g., antibody-heavy and light chains) by brute-force screening. This is adequate for the pharmaceutical industry because the cost of this method is small compared to that of a clinical trial. However, more complex systems will require faster, more reproducible technologies for engineering mammalian cells.

There has been some success in engineering genetic circuits in mammalian cells. As with bacteria, much of the effort has focused on gene expression-based devices (Deans et al., 2007). Transcriptional logic-based circuits analogous to those constructed in bacteria have also been engineered into

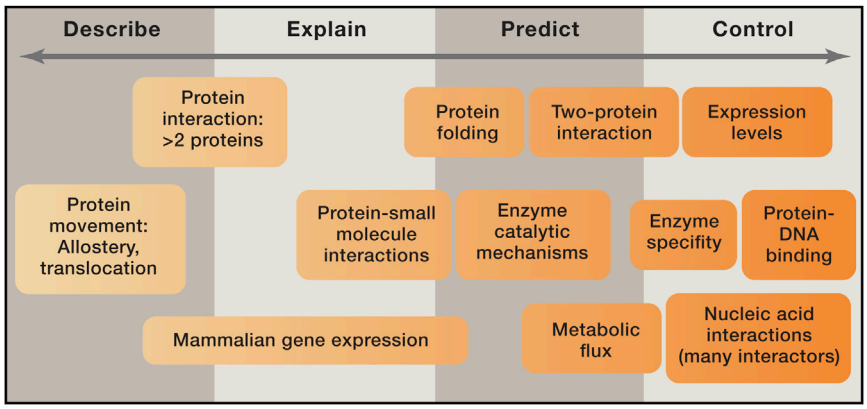

Figure 3. Biological Understanding along the "Describe-Explain-Predict-Control" Axis
The figure diagrams a rough estimate of how well we understand and can engineer various biomolecular elements and events. Some, such as nucleic acid interactions with other nucleic acids and certain proteins, are so well understood that they can be predictably engineered with minimal postbuild testing. Others, such as movement of proteins during allosteric transitions or along cytoskeletal elements, can be described and their behavior sometimes rationalized post hoc, but to engineer such systems, it is often difficult even to know where to start. Fully controlling the remarkable capabilities of cells and organisms will require integrated engineering of the extensive phenomena of biology.

mammalian cells, but there are also challenges in construct assembly still being addressed (Wieland and Fussenegger, 2012; Wei et al., 2013; Guye et al., 2013). For example, Burrill et al. (2012) constructed memory systems in mammalian cells, in which trigger and memory elements were sequentially transfected into cells. A single trigger element construct was inserted randomly into the genome by standard procedures, and clones were screened for the ability to respond to an inducing signal (doxycycline, DNA damage, and hypoxia); clones with the correct behavior were then transfected with a memory element and screened for the ability to retain a memory of the inducing signal. In this approach, quantitative adjustment of the promoter and ribosome-binding site (RBS) strength was replaced by varying the insertion site and letting the influence of local chromatin structure determine the level of gene expression. This approach does not fulfill the desire for precise and predictable engineering of mammalian cells. Recently, new tools have emerged that should allow specific integration at desired sites in the genome. For example, methods based on zinc-finger, TALE, and CRISPR fusions to nucleases can be used to generate double-strand breaks at specific sites in the genome (Gaj et al., 2013). The questions remain—where should we integrate, and how can we avoid effects of adjacent sequences?

What is needed to make mammalian cells engineerable? Scientific understanding can be oriented along an axis of "describe-explain-predict-control" (Evans and Rooney, 2008; Figure 3). Elements of bacterial gene expression lie toward the "control" side of this continuum, but gene expression in multicellular organisms is still somewhere in the middle. A variety of elements that are important in metazoan gene expression have been identified, including enhancers, TATA elements, silencers, and insulators, but it is not clear how to combine these elements to create new regulatory systems with quantitatively predictable properties. Many of these elements are understood in some mechanistic detail, but this information is distributed throughout the scientific literature, making it difficult to extract the key bits of information that are useful for engineering. A particular issue that is somewhat unique to mammalian cells is that long-range genomic context effects have a profound effect on expression of transgenes (Carroll et al., 2005; Ribeiro de Almeida et al., 2012). It is difficult to simply look (even with computers) at a genome sequence and figure out where the enhancers, insulators, and sometimes even coding sequences are located. In addition, the long-range nature of metazoan transcriptional control means that even transgenes may interfere with each other. For effective, predictable engineering of mammalian cells, it may be necessary to construct a well-understood artificial chassis chromosome. Such an element would need to have defined insertion sites, insulators that functionally separate transgenes from each other, and physical separation from the native genome to avoid unpredictable long-range effects. There has been some effort to construct artificial chromosomes "top-down" by deletion of material from an existing chromosome (Ren et al., 2006; Bergmann et al., 2012), but remaining undeleted material may still influence expression of inserted transgenes. Piecing together all of the elements of an artificial chromosome by a "bottom-up" approach is possible in principle but would require that we know all of the pieces. Hopefully, one or both of these approaches will bear fruit, because the need for a mammalian chassis chromosome is critical.

Mammalian signal transduction can also be engineered. Yeh et al. (2007) modified a signal transduction pathway that controls the actin cytoskeleton to alter cell shape in response to an external signal, a response that did not involve gene expression. Several groups have also engineered T cells to recognize tumor cells by expression of chimeric antigen receptors (CARs; Gross et al., 1989; Porter et al., 2011). These elements consist of single-chain antibody V regions fused to the ζ chain of the T cell receptor; the V regions recognize tumor-specific surface antigens.

The CAR construction is a synthetic-biological creation with an obvious practical utility. As such, it evokes the words of Steve Jobs (Feld, 2011): "You've got to start with the customer experience and work backwards to the technology. You can't start with the technology and try to figure out where you're going to try to sell it." The creators of CARs started with a particular "customer experience" goal, treating cancer, and then developed a technology for doing so. Development of CARs did not appear to begin with design specifications beyond wanting to kill cancer cells; such specifications might have included a therapeutic index that could be optimized by adjusting the binding constant of the antibody for its targets. Nonetheless, the approach works at some level in leukemias in which the target cells are accessible (Porter et al., 2011), and quantitative optimization may help when the antigen is not completely tumor

specific or for solid tumors in which the cells are less accessible. The CAR example illustrates the value of engineering signaling proteins that are far removed from transcription, even though we do not have particularly elegant tools for doing so.

Designing to Meet Biological Grand Challenges

In sum, the original guiding principles conceptualized for synthetic biology a decade ago have permeated a widespread, diverse community, but it remains to be seen which elements merit adoption as research becomes more translational. For this to happen, it is worth considering synthetic biology approaches in light of the challenges posed at the beginning of this Review: designing microbes that produce biofuels that can go directly into a gas tank; developing immune cells that kill metastatic solid tumor cells; and creating engineered food crops with increased per acre yield and reduced fertilizer and water use. Can synthetic biology deliver these products? Following Steve Jobs, let us look ahead to the final product that we want and then work backward to the design specifications.

For living cells to make biofuels, the specifications might be that the fuel molecules would spontaneously separate from the aqueous phase and from cell membranes, would not be toxic to the cells, would have appropriate combustion properties, and would be efficiently produced, etc. To have appropriate combustion properties, it is likely necessary to synthesize a mixture of molecules similar to fuels from petroleum feedstocks, such as a mix of linear and branched medium-length alkanes, isoprenoids, and related molecules. To efficiently synthesize the medium-length linear alkanes, one could modify fatty acid synthesis such that it terminates before the usual C16- to C18-length molecules and express enzymes to make alkanes or fatty alcohols (Schirmer et al., 2010; Steen et al., 2010) but with shorter-than-usual length specificity, so extensive protein engineering would be required. In addition, the producing cell would need to make normal fatty acids to grow and then switch on production of the alternative pathway, while switching off synthesis of full-length fatty acids and possibly cell growth itself to minimize the waste of carbon feedstock. To insure correct distribution of acetyl-CoA into fatty acid initiation and elongation, it may be necessary to adjust the natural mechanism to reflect the shorter chain length of the desired product. Coexpression of certain amino acid and isoprenoid pathways would yield a useful mix of branched carbon chains. Thus, the final engineered organism would require both extensive protein and transcriptional engineering and, possibly, engineering of the novel enzymes into a scaffolded structure to enhance synthesis of the specific product. All of these engineering modes would need to be tightly interdigitated.

The design specifications for engineered immune cells that successfully attack solid tumors would be that enough metastatic tumor cells are killed to cause a complete remission possibly with lasting immunity, that the side effects are tolerable, and that systemic autoimmunity does not result. The challenges for any immunological therapy to treat solid tumors are formidable. Unlike leukemias and lymphomas, solid tumor cells will be separated from the engineered T cells by a barrier of capillary endothelial cells and a lack of lymph node drainage to draw anything into the tumor. The T cells might first need to be engineered to recognize the tumor-specific endothelium with coupling to the system that mediates tissue invasion. Once in the tumor, the T cells would specifically recognize and kill the tumor cells, in spite of the numerous immunosuppressive cytokines being secreted by the tumor. The number of cells killed per attacking T cells must likely be higher than normal, so the mechanisms for downregulating T cell killing would need to be modified, but not in a way that would allow attack on normal cells. Finally, the engineered cells would need to die or be easily killed after the tumor is eliminated. Such engineering could build on the CAR-expressing T cells but would be several times more complicated. The behavior of solid tumors in humans is different than in mouse models because the human tumors grow more slowly, and some underlying immunology is simply different between the two organisms (Mestas and Hughes, 2004). Therefore the design may need to be particularly theory driven, with limited experimental support before a Phase 1 clinical trial. This truly would resemble a moon shot—the calculations would need to be right.

The increases in the per-acre yields of food crops have been flattening over the past 15 years or so, after dramatic increases of up to 6-fold that occurred between the 1960s and 1990s (Fischer et al., 2009). The S-shaped curve for crop yields contrasts with the exponential growth of the human population, and new sources of food will be needed. The green revolution combined traditional crop breeding with intensified water and fertilizer use, which led to increased crop yields but with an environmentally unsustainable approach, particularly in its use of nitrogeous fertilizers. One design specification would be that plants simply fix their own nitrogen. This would presumably involve introduction of a large number of genes from N_2-fixing bacteria, plus regulation and compartmentalization so that the genes are expressed in an oxygen-free environment.

Conclusions

Synthetic biology is still in its early stages of development. If we stick with the comparison to the microchip industry and consider that the first transistor was developed in 1947, then we are now at about 1960. In the near future, we also hope that the approaches of synthetic biology will have permeated the biological community. In particular, the most important engineering ideas—design to specification and extensive pre-experiment planning to avoid excessive trial and error—need to be adopted because it should be more cost effective than the current style. For synthetic biology to go beyond a self-selected community, it will need successes with clear-cut utility that clearly derive from the field's specific approach.

If synthetic biologists are to harness the power of biology, they will need to have a command of all the things that biology modulates during the course of evolution—e.g., gene expression, metabolism, protein structure, and function. Nature manipulates all of these things at the same time, and synthetic biologists need to be able to do so as well. The redesign of biology will necessarily involve an integration multifaceted engineering skills.

This leads to a key question for the future development of synthetic biology: do human engineers need to understand biology as it exists with all of its nonintuitive quirkiness, or can biology be redesigned so that engineers work with a simplified form that is

adapted to the creative processes of the human brain? In the case of computers, the latter is the case—materials scientists have adapted inorganic matter to be used in transistors in a way that is physically robust and also abstractable. Unfortunately, as of now, the approaches of synthetic biology do not always allow for a single solution that can be used over and over again. Moreover, some accomplishments of nature are hard for humans to reproduce—for example, protein engineers have not yet built de-novo-designed proteins, particularly enzymes, that are truly useful in biological engineering. It therefore seems likely that most synthetic biology will involve repeated, intensive borrowing from nature, and the most effective practitioners in the further development of the field will be those with an engineering mindset who, functioning alone or in integrated teams, understand most broadly how natural biology works.

ACKNOWLEDGMENTS

We acknowledge the Wyss Institute for continued support, the original Synthetic Biology Working Group at MIT, the many participants in iGEM since its inception, past and present members of our laboratories, a few visionary government scientists and program managers from DARPA and ONR who appreciated the potential of the field before it existed, and Devin Burrill for comments on the manuscript. The authors acknowledge support over the years from DARPA, ONR, HHMI, DOE, NIH, NSF, and their respective institutions.

REFERENCES

Ackers, G.K., Johnson, A.D., and Shea, M.A. (1982). Quantitative model for gene regulation by lambda phage repressor. Proc. Natl. Acad. Sci. USA *79*, 1129–1133.

Agapakis, C.M., Ducat, D.C., Boyle, P.M., Wintermute, E.H., Way, J.C., and Silver, P.A. (2010). Insulation of a synthetic hydrogen metabolism circuit in bacteria. J. Biol. Eng. *4*, 3.

Ajo-Franklin, C.M., Drubin, D.A., Eskin, J.A., Gee, E.P., Landgraf, D., Phillips, I., and Silver, P.A. (2007). Rational design of memory in eukaryotic cells. Genes Dev. *21*, 2271–2276.

Albert, R., Collins, J.J., and Glass, L. (2013). Introduction to focus issue: quantitative approaches to genetic networks. Chaos *23*, 025001.

Arkin, A., Ross, J., and McAdams, H.H. (1998). Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. Genetics *149*, 1633–1648.

Atsumi, S., Hanai, T., and Liao, J.C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature *451*, 86–89.

Backman, K., and Ptashne, M. (1978). Maximizing gene expression on a plasmid using recombination in vitro. Cell *13*, 65–71.

Bagshawe, K.D. (2009). Targeting: the ADEPT story so far. Curr. Drug Targets *10*, 152–157.

Bergmann, J.H., Martins, N.M., Larionov, V., Masumoto, H., and Earnshaw, W.C. (2012). HACking the centromere chromatin code: insights from human artificial chromosomes. Chromosome Res. *20*, 505–519.

Bomford, P. (2009). Crop yield projections for biofuels fall short. Post Carbon Institute, September 4, 2009. http://www.postcarbon.org/article/40439-crop-yield-projections-for-biofuels-fall.

Bonnet, J., Subsoontorn, P., and Endy, D. (2012). Rewritable digital data storage in live cells via engineered control of recombination directionality. Proc. Natl. Acad. Sci. USA *109*, 8884–8889.

Bonnet, J., Yin, P., Ortiz, M.E., Subsoontorn, P., and Endy, D. (2013). Amplifying genetic logic gates. Science *340*, 599–603.

Bork, P., Schultz, J., and Ponting, C.P. (1997). Cytoplasmic signalling domains: the next generation. Trends Biochem. Sci. *22*, 296–298.

Bultmann, S., Morbitzer, R., Schmidt, C.S., Thanisch, K., Spada, F., Elsaesser, J., Lahaye, T., and Leonhardt, H. (2012). Targeted transcriptional activation of silent oct4 pluripotency gene by combining designer TALEs and inhibition of epigenetic modifiers. Nucleic Acids Res. *40*, 5368–5377.

Burrill, D.R., and Silver, P.A. (2011). Synthetic circuit identifies subpopulations with sustained memory of DNA damage. Genes Dev. *25*, 434–439.

Burrill, D.R., Inniss, M.C., Boyle, P.M., and Silver, P.A. (2012). Synthetic memory circuits for tracking human cell fate. Genes Dev. *26*, 1486–1497.

Callura, J.M., Dwyer, D.J., Isaacs, F.J., Cantor, C.R., and Collins, J.J. (2010). Tracking, tuning, and terminating microbial physiology using synthetic riboregulators. Proc. Natl. Acad. Sci. USA *107*, 15898–15903.

Carlson, R. (2009). The changing economics of DNA synthesis. Nat. Biotechnol. *27*, 1091–1094.

Carroll, J.S., Liu, X.S., Brodsky, A.S., Li, W., Meyer, C.A., Szary, A.J., Eeckhoute, J., Shao, W., Hestermann, E.V., Geistlinger, T.R., et al. (2005). Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell *122*, 33–43.

Casadaban, M.J. (1976). Transposition and fusion of the lac genes to selected promoters in Escherichia coli using bacteriophage lambda and Mu. J. Mol. Biol. *104*, 541–555.

Cheng, S., Liu, Y., Crowley, C.S., Yeates, T.O., and Bobik, T.A. (2008). Bacterial microcompartments: their properties and paradoxes. Bioessays *30*, 1084–1095.

Chester, J., Ruchatz, A., Gough, M., Crittenden, M., Chong, H., Cosset, F.L., Diaz, R.M., Harrington, K., Alvarez-Vallina, L., and Vile, R. (2002). Tumor antigen-specific induction of transcriptionally targeted retroviral vectors from chimeric immune receptor-modified T cells. Nat. Biotechnol. *20*, 256–263.

Cironi, P., Swinburne, I.A., and Silver, P.A. (2008). Enhancement of cell type specificity by quantitative modulation of a chimeric ligand. J. Biol. Chem. *283*, 8469–8476.

Deans, T.L., Cantor, C.R., and Collins, J.J. (2007). A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. Cell *130*, 363–372.

Delebecque, C.J., Lindner, A.B., Silver, P.A., and Aldaye, F.A. (2011). Organization of intracellular reactions with rationally designed RNA assemblies. Science *333*, 470–474.

Dharmadi, Y., Patel, K., Shapland, E., Hollis, D., Slaby, T., Klinkner, N., Dean, J., and Chandran, S.S. (2013). High-throughput, cost-effective verification of structural DNA assembly. Nucleic Acids Res.. http://dx.doi.org/10.1093/nar/gkt1088.

Douglas, S.M., Marblestone, A.H., Teerapittayanon, S., Vazquez, A., Church, G.M., and Shih, W.M. (2009). Rapid prototyping of 3D DNA-origami shapes with caDNAno. Nucleic Acids Res. *37*, 5001–5006.

Dueber, J.E., Wu, G.C., Malmirchegini, G.R., Moon, T.S., Petzold, C.J., Ullal, A.V., Prather, K.L., and Keasling, J.D. (2009). Synthetic protein scaffolds provide modular control over metabolic flux. Nat. Biotechnol. *27*, 753–759.

Elowitz, M.B., and Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. Nature *403*, 335–338.

Elrod-Erickson, M., Benson, T.E., and Pabo, C.O. (1998). High-resolution structures of variant Zif268-DNA complexes: implications for understanding zinc finger-DNA recognition. Structure *6*, 451–464.

Endy, D., Lincoln, P., and Murray, R. (2003). 2003 Synthetic Biology Study. http://hdl.handle.net/1721.1/38455.

Evans, A.N., and Rooney, B.J. (2008). Methods in Psychological Research (Thousand Oaks, CA: Sage Publications).

Feist, A.M., and Palsson, B.O. (2008). The growing scope of applications of genome-scale metabolic reconstructions using Escherichia coli. Nat. Biotechnol. *26*, 659–667.

Feld, B. (2011) Start with customer experience. http://www.feld.com/wp/archives/2011/09/start-with-customer-experience.html.

Fischer, R.A., Byerlee, E., and Edmeades, E.O. (2009). Can technology deliver on the yield challenge to 2050? ftp://ftp.fao.org/docrep/fao/012/ak977e/ak977e00.pdf.

Friedland, A.E., Lu, T.K., Wang, X., Shi, D., Church, G., and Collins, J.J. (2009). Synthetic gene networks that count. Science *324*, 1199–1202.

Gaj, T., Gersbach, C.A., and Barbas, C.F., 3rd. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. *31*, 397–405.

Gardner, T.S., and Hawkins, K. (2013). Synthetic biology: evolution or revolution? A co-founder's perspective. Curr. Opin. Chem. Biol. *17*, 871–877.

Gardner, T.S., Cantor, C.R., and Collins, J.J. (2000). Construction of a genetic toggle switch in Escherichia coli. Nature *403*, 339–342.

Garg, A., Lohmueller, J.J., Silver, P.A., and Armel, T.Z. (2012). Engineering synthetic TAL effectors with orthogonal target sites. Nucleic Acids Res. *40*, 7584–7595.

Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A., 3rd, and Smith, H.O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods *6*, 343–345.

Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., Algire, M.A., Benders, G.A., Montague, M.G., Ma, L., Moodie, M.M., et al. (2010). Creation of a bacterial cell controlled by a chemically synthesized genome. Science *329*, 52–56.

Gilbert, W. (1978). Why genes in pieces? Nature *271*, 501.

Glass, J.I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M.R., Maruf, M., Hutchison, C.A., 3rd, Smith, H.O., and Venter, J.C. (2006). Essential genes of a minimal bacterium. Proc. Natl. Acad. Sci. USA *103*, 425–430.

Goodman, D.B., Church, G.M., and Kosuri, S. (2013). Causes and effects of N-terminal codon bias in bacterial genes. Science *342*, 475–479.

Gross, G., Waks, T., and Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc. Natl. Acad. Sci. USA *86*, 10024–10028.

Guntas, G., Mansell, T.J., Kim, J.R., and Ostermeier, M. (2005). Directed evolution of protein switches and their application to the creation of ligand-binding proteins. Proc. Natl. Acad. Sci. USA *102*, 11224–11229.

Guye, P., Li, Y., Wroblewska, L., Duportet, X., and Weiss, R. (2013). Rapid, modular and reliable construction of complex mammalian gene circuits. Nucleic Acids Res. *41*, e156.

Hutchison, C.A., Peterson, S.N., Gill, S.R., Cline, R.T., White, O., Fraser, C.M., Smith, H.O., and Venter, J.C. (1999). Global transposon mutagenesis and a minimal Mycoplasma genome. Science *286*, 2165–2169.

Huynen, M.A., and Bork, P. (1998). Measuring genome evolution. Proc. Natl. Acad. Sci. USA *95*, 5849–5856.

Isaacs, F.J., Dwyer, D.J., Ding, C., Pervouchine, D.D., Cantor, C.R., and Collins, J.J. (2004). Engineered riboregulators enable post-transcriptional control of gene expression. Nat. Biotechnol. *22*, 841–847.

Kahl, L.J., and Endy, D. (2013). A survey of enabling technologies in synthetic biology. J. Biol. Eng. *7*, 13.

Keasling, J.D. (2012). Synthetic biology and the development of tools for metabolic engineering. Metab. Eng. *14*, 189–195.

Keasling, J.D., Mendoza, A., and Baran, P.S. (2012). Synthesis: A constructive debate. Nature *492*, 188–189.

Khalil, A.S., Lu, T.K., Bashor, C.J., Ramirez, C.L., Pyenson, N.C., Joung, J.K., and Collins, J.J. (2012). A synthetic biology framework for programming eukaryotic transcription functions. Cell *150*, 647–658.

Knight, T.F. (2003). Idempotent vector design for standard assembly of BioBricks. MIT Synthetic Biology Working Group Technical Reports. ([http://hdl.handle.net/1721.1/21168]).

Kobayashi, H., Kaern, M., Araki, M., Chung, K., Gardner, T.S., Cantor, C.R., and Collins, J.J. (2004). Programmable cells: interfacing natural and engineered gene networks. Proc. Natl. Acad. Sci. USA *101*, 8414–8419.

Kosuri, S., Eroshenko, N., Leproust, E.M., Super, M., Way, J., Li, J.B., and Church, G.M. (2010). Scalable gene synthesis by selective amplification of DNA pools from high-fidelity microchips. Nat. Biotechnol. *28*, 1295–1299.

Kosuri, S., Goodman, D.B., Cambray, G., Mutalik, V.K., Gao, Y., Arkin, A.P., Endy, D., and Church, G.M. (2013). Composability of regulatory sequences controlling transcription and translation in Escherichia coli. Proc. Natl. Acad. Sci. USA *110*, 14024–14029.

Kung, Y., Runguphan, W., and Keasling, J.D. (2012). From fields to fuels: recent advances in the microbial production of biofuels. ACS Synth. Biol. *1*, 498–513.

Lajoie, M.J., Rovner, A.J., Goodman, D.B., Aerni, H.R., Haimovich, A.D., Kuznetsov, G., Mercer, J.A., Wang, H.H., Carr, P.A., Mosberg, J.A., et al. (2013). Genomically recoded organisms expand biological functions. Science *342*, 357–360.

Lienert, F., Torella, J.P., Chen, J.H., Norsworthy, M., Richardson, R.R., and Silver, P.A. (2013). Two- and three-input TALE-based AND logic computation in embryonic stem cells. Nucleic Acids Res. *41*, 9967–9975.

Mathews, C.K. (1993). The cell-bag of enzymes or network of channels? J. Bacteriol. *175*, 6377–6381.

Meister, G.E., and Joshi, N.S. (2013). An engineered calmodulin-based allosteric switch for Peptide biosensing. ChemBioChem *14*, 1460–1467.

Merrihew, R.V., Marburger, K., Pennington, S.L., Roth, D.B., and Wilson, J.H. (1996). High-frequency illegitimate integration of transfected DNA at preintegrated target sites in a mammalian genome. Mol. Cell. Biol. *16*, 10–18.

Mestas, J., and Hughes, C.C. (2004). Of mice and not men: differences between mouse and human immunology. J. Immunol. *172*, 2731–2738.

Moon, T.S., Yoon, S.H., Lanza, A.M., Roy-Mayhew, J.D., and Prather, K.L. (2009). Production of glucaric acid from a synthetic pathway in recombinant Escherichia coli. Appl. Environ. Microbiol. *75*, 589–595.

Murnane, J.P., Yezzi, M.J., and Young, B.R. (1990). Recombination events during integration of transfected DNA into normal human cells. Nucleic Acids Res. *18*, 2733–2738.

Mutalik, V.K., Guimaraes, J.C., Cambray, G., Mai, Q.A., Christoffersen, M.J., Martin, L., Yu, A., Lam, C., Rodriguez, C., Bennett, G., et al. (2013a). Quantitative estimation of activity and quality for collections of functional genetic elements. Nat. Methods *10*, 347–353.

Mutalik, V.K., Guimaraes, J.C., Cambray, G., Lam, C., Christoffersen, M.J., Mai, Q.A., Tran, A.B., Paull, M., Keasling, J.D., Arkin, A.P., and Endy, D. (2013b). Precise and reliable gene expression via standard transcription and translation initiation elements. Nat. Methods *10*, 354–360.

Oberhardt, M.A., Palsson, B.O., and Papin, J.A. (2009). Applications of genome-scale metabolic reconstructions. Mol. Syst. Biol. *5*, 320.

Porter, D.L., Levine, B.L., Kalos, M., Bagg, A., and June, C.H. (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N. Engl. J. Med. *365*, 725–733.

Rader, R.A. (2012). FDA biopharmaceutical product approvals and trends: 2011. http://www.biopharma.com/approvals_2011.html.

Ren, X., Tahimic, C.G., Katoh, M., Kurimasa, A., Inoue, T., and Oshimura, M. (2006). Human artificial chromosome vectors meet stem cells: new prospects for gene delivery. Stem Cell Rev. *2*, 43–50.

Ribeiro de Almeida, C., Stadhouders, R., Thongjuea, S., Soler, E., and Hendriks, R.W. (2012). DNA-binding factor CTCF and long-range gene interactions in V(D)J recombination and oncogene activation. Blood *119*, 6209–6218.

Robinson-Mosher, A., Shinar, T., Silver, P.A., and Way, J. (2013). Dynamics simulations for engineering macromolecular interactions. Chaos *23*, 025110.

Salis, H.M. (2011). The ribosome binding site calculator. Methods Enzymol. *498*, 19–42.

Schirmer, A., Rude, M.A., Li, X., Popova, E., and del Cardayre, S.B. (2010). Microbial biosynthesis of alkanes. Science *329*, 559–562.

Sharma, S.S., Blattner, F.R., and Harcum, S.W. (2007). Recombinant protein production in an Escherichia coli reduced genome strain. Metab. Eng. *9*, 133–141.

Shetty, R.P., Endy, D., and Knight, T.F., Jr. (2008). Engineering BioBrick vectors from BioBrick parts. J. Biol. Eng. *14*, 5.

Silhavy, T.J., Casadaban, M.J., Shuman, H.A., and Beckwith, J.R. (1976). Conversion of beta-galactosidase to a membrane-bound state by gene fusion. Proc. Natl. Acad. Sci. USA *73*, 3423–3427.

Siuti, P., Yazbek, J., and Lu, T.K. (2013). Synthetic circuits integrating logic and memory in living cells. Nat. Biotechnol. *31*, 448–452.

Steen, E.J., Kang, Y., Bokinsky, G., Hu, Z., Schirmer, A., McClure, A., Del Cardayre, S.B., and Keasling, J.D. (2010). Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature *463*, 559–562.

Tinberg, C.E., Khare, S.D., Dou, J., Doyle, L., Nelson, J.W., Schena, A., Jankowski, W., Kalodimos, C.G., Johnsson, K., Stoddard, B.L., and Baker, D. (2013). Computational design of ligand-binding proteins with high affinity and selectivity. Nature *501*, 212–216.

Wang, H.H., Isaacs, F.J., Carr, P.A., Sun, Z.Z., Xu, G., Forest, C.R., and Church, G.M. (2009). Programming cells by multiplex genome engineering and accelerated evolution. Nature *460*, 894–898.

Wei, K.Y., Chen, Y.Y., and Smolke, C.D. (2013). A yeast-based rapid prototype platform for gene control elements in mammalian cells. Biotechnol. Bioeng. *110*, 1201–1210.

Whitaker, W.R., and Dueber, J.E. (2011). Metabolic pathway flux enhancement by synthetic protein scaffolding. Methods Enzymol. *497*, 447–468.

Wieland, M., and Fussenegger, M. (2012). Engineering molecular circuits using synthetic biology in mammalian cells. Annu. Rev. Chem. Biomol. Eng. *3*, 209–234.

Win, M.N., Liang, J.C., and Smolke, C.D. (2009). Frameworks for programming biological function through RNA parts and devices. Chem. Biol. *16*, 298–310.

Yeh, B.J., Rutigliano, R.J., Deb, A., Bar-Sagi, D., and Lim, W.A. (2007). Rewiring cellular morphology pathways with synthetic guanine nucleotide exchange factors. Nature *447*, 596–600.

Young, R., and Bremer, H. (1976). Polypeptide-chain-elongation rate in Escherichia coli B/r as a function of growth rate. Biochem. J. *160*, 185–194.