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Current Opinion in Chemical Biology

Advances in genetic circuit design: novel biochemistries, deep part mining, and precision gene expression

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Cells use regulatory networks to perform computational operations to respond to their environment. Reliably manipulating such networks would be valuable for many applications in biotechnology; for example, in having genes turn on only under a defined set of conditions or implementing dynamic or temporal control of expression. Still, building such synthetic regulatory circuits remains one of the most difficult challenges in genetic engineering and as a result they have not found widespread application. Here, we review recent advances that address the key challenges in the forward design of genetic circuits. First, we look at new design concepts, including the construction of layered digital and analog circuits, and new approaches to control circuit response functions. Second, we review recent work to apply part mining and computational design to expand the number of regulators that can be used together within one cell. Finally, we describe new approaches to obtain precise gene expression and to reduce context dependence that will accelerate circuit design by more reliably balancing regulators while reducing toxicity.

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Current Opinion in Chemical Biology 2013, **17**:878–892

This review comes from a themed issue on **Synthetic biology**

Edited by **Adam P Arkin** and **Martin Fussenegger**

For a complete overview see the Issue and the Editorial

Available online 19th November 2013

1367-5931/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.cbpa.2013.10.003

Introduction

Cells naturally control gene expression using a variety of RNA, protein, and DNA-modifying regulators [1–3]. It was recognized early on that interactions between these regulators could lead to computational operations that are analogous to electronic circuits [4–6]. Genetic engineers have since attempted to build synthetic circuits that would implement artificial programs of gene expression. This could have a revolutionary impact on biotechnology, such as programming bacteria to individually respond to transient conditions in a bioreactor [7], designing therapeutic cells to sense and respond to diseased states within the human body [8–12], or engineering smart plants that can respond to conditions in the environment [13]. However, building synthetic circuits remains one of the greatest challenges in the field, where even simple circuitry is labor intensive to build and lacks the performance of its natural counterparts. As a result, synthetic genetic circuits have been slow to appear in biotechnology applications [14].

There are several reasons why genetic circuit design has been challenging compared to other areas in genetic engineering. First, circuits require precise tuning in the expression levels of their component regulators [15]. This is less essential when engineering cells to make small molecules or individual proteins, where genes tend to be maximally expressed. Second, regulators are prone to being toxic and, even when slight, this can inhibit growth and lead to evolutionary instability and a reduction in strain performance. Third, the regulatory interactions within a circuit all occur within the cell and crosstalk between them or with the host can impact the desired circuit behavior [16]. Fourth, there are few design rules for the systematic improvement of circuit performance (speed, dynamic range, robustness, and cell-to-cell variability). Finally, the physical construction of circuits requires the assembly of many parts, which until recently, has been technically challenging [17–19]. Often, these parts appear in genetic contexts that are different than that in which they were characterized and this can lead to interference [7].

In this review, we focus on recent advances in synthetic circuit design for bacteria. There have been other reviews looking at circuit design for eukaryotes and higher organisms [14,20,22–24]. We have divided the review into three sections. In the first section, we describe recent advances in different types of circuit design. Next, we describe how the toolbox of regulators has expanded, both in increasing the number of characterized regulators from different families, as well as the discovery of new biochemistries that can be harnessed for synthetic regulatory networks. Finally, we review new approaches to obtain precision expression control and its potential impact on building sophisticated circuitry.

I. Advanced circuit designs

To date, most of the genetic circuits that have been constructed are so small that there has been little need to utilize advanced concepts or algorithms in their design. As they get more sophisticated, however, it will become more difficult to identify a pattern of regulatory interactions that can produce a desired function. To this end,

approaches from electrical digital and analog circuit design have begun to be applied. Realizing these designs requires that regulators be functionally connected. This will require better control over their response functions (the input-output relationship), as well as handling other circuit characteristics such as retroactivity and evolutionary instability.

Layered digital circuits

Digital circuits produce signals at discrete levels (most commonly, high and low or 1 and 0), as opposed to operating in a continuous range. Their advantage is in their designability; there are many design tools that can abstract a desired circuit function into a large assembly of logic gates [25]. This designability comes at the cost of size and power requirements. Many digital gates may be needed to produce a computational function compared to what would be required if continuous variables were allowed. In terms of genetic circuits, this manifests as more DNA, regulators, and energetic resource expenditure [26–28].

Many genetic circuits have been built that produce Boolean logic functions, or 'logic gates' [29–31]. Note that while these are often described as being digital, all of these circuits exhibit analog features (so-called fuzzy logic), where there is a continuous change in output. This can be used as the basis for the construction of analog circuitry (next section).

If genetic logic gates are designed to have inputs and outputs that have the same signal, they can be layered to produce more complex computational operations. In practice, this signal is transcriptionl activity, where the inputs and outputs are promoters. This approach is modular but slow, with each layer requiring a step of transcription and translation with a timescale of 20 min [32]. Further, if one of the signals skips a layer, this can produce a fault where the output is transiently incorrect. Such faults have been exploited in the construction of pulse-generating genetic circuits, in the form of incoherent feed-forward loops [33–35].

There have been several studies to layer logic gates to produce more complex functions. This is closely related to work to build cascades through the connection of gates in a linear series [32,36,37]. As a proof-of-principle, a 4-input AND gate was built by layering three 2-input AND gates along with additional layers that contain the four sensors and an output [28] (Figure 1(a)). It has also been shown that a set of orthogonal NOR gates can be layered to form different logic operations by permuting the input and output promoters to produce different wiring diagrams [38]. Both of these examples perform relatively simple computations that could be designed by hand and both required more gates than would be needed using other types of regulators. It would require significantly larger circuits to realize the benefits of layered digital gates and computational design automation [25,39,40•].

Analog circuits

Analog circuits operate with continuous signals. In electronic circuits, they are used when there are limitations in the number of components or power that can be used (e.g., in medical devices) [41]. This comes at a cost of designability, where each circuit has to be individually designed and simulated, which limits the size and flexibility of the circuits. In practice, every genetic circuit — natural or synthetic — is analog to some degree and this needs to be accounted for in their design. The question is to what extent the design of genetic circuits can benefit from the principles used for building analog electronic circuits.

The value in considering analog circuit design was recently demonstrated [42••]. The authors of the study were able to implement circuits that computed mathematical functions that would otherwise require many digital gates, including logarithm and power-law functions, and continuous addition and division (Figure 1(b)). Their circuit generates a wide dynamic range response function using a positive feedback loop and a second promoter to titrate away the activator. This response computes the logarithm of inducer concentration and the introduction of a second positive feedback loop produces a circuit that computes log-domain addition of two inducers. A log-domain division circuit was further engineered by having the two feedback loops compute the ratio between the two inducers. Remarkably, all of these arithmetic functions were computed using only two transcription factors.

Recombinase-based memory and logic

Logic gates based on transcription factors often exhibit analog features, with graded switch transitions. In contrast, highly digital switches can be built using recombinases that catalyze a sequence-specific change in the orientation of a unit of DNA, where each orientation corresponds to a different signal level. Recombinases have been used as the basis for a number of synthetic circuits [43–45] and have been layered to form a cascade [46]. Previously, the recombinases used were either irreversible (where the inversion is unidirectional) or reversible (where the same recombinase catalyzes both directions). Recently, a rewriteable switch has been built based on a system where an integrase catalyzes the switch in one direction and an integrase/excisionase pair catalyzes the reverse reaction [47••]. This is a significant improvement in that it allows the signal to both hold permanently and also switch back to the initial state.

Multiple recombinases have been built into circuits that function as 'memory logic' devices, where the rearrangement of DNA is triggered by two input inducers [48•,49].