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NEWS AND VIEWS

Cell-free synthetic biology: a bottom-up approach to discovery by design

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The practitioners of the brand of synthetic biology that lies closest to engineering are fond of quoting the phrase Richard Feynman had written on his blackboard: 'What I cannot create I do not understand'. This phrase captures both a warning about the limitations of analysis in understanding complex systems and an endorsement of the value of design in the quest for discovery. Analysis, modeling, and simulation have a tendency to focus attention on the details of individual elements and components, whereas the reiterative nature of design requires grappling with the trade-offs and compromises required to enable system function. The emergence of this system view from design suggests that redesign (or rewriting in the context of evolved systems) is a promising route for understanding the fundamental principles governing the organization of natural genetic systems. Synthetic biology rewriting efforts have usually followed a strategy of constructing deliberately simplified systems to build an understanding of cellular regulatory processes from the bottom-up (Hasty *et al*, 2002; Sprinzak and Elowitz, 2005; Andrianantoandro *et al*, 2006; Guido *et al*, 2006). Although such rewriting efforts are best served when every interconnection of components is a design choice, the synthetic gene circuits deployed within cells are supported by the global resources of the cell and are subject to unintended interactions such as the coupling of extrinsic noise between otherwise unconnected gene circuits (Pedraza and van Oudenaarden, 2005) or inducer interference with non-transcriptional processes (Austin *et al*, 2006). A more bottom-up approach to the rewriting of genetic systems is reported (Kim *et al*, 2006) where a cell-free strategy minimizes unintended interactions by eliminating cellular support of synthetic gene circuits (Figure 1).

In addition to going cell-free, the Kim *et al* approach further simplifies rewriting by reducing gene expression to the single step of transcription. Transcriptional regulation is performed

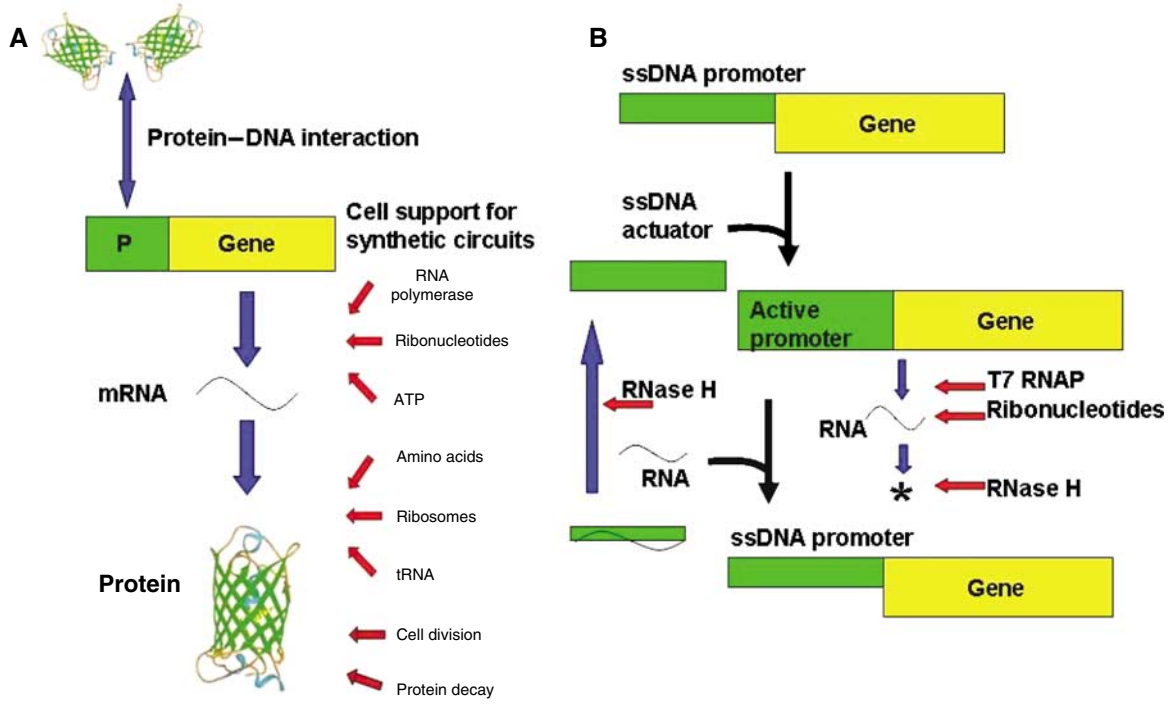

Figure 1 Comparison of whole-cell and cell-free synthetic biology. (**A**) Global cellular resources support the operation of synthetic circuits in whole-cell synthetic biology, but also provide subtle pathways for interactions between otherwise unconnected gene circuits. Much of transcriptional regulation is achieved through protein–DNA interactions. (**B**) The cell-free synthetic biology approach reported by Kim *et al* removes global resources to support gene circuit operation, translation, and protein transcription factors. Transcriptional regulation is achieved by the competing actions of single-stranded DNA activators and complementary strands of RNA.

by the resulting RNA molecules with specificity provided by complementary base-pair formation rather than by protein–DNA interactions. Such an approach has precedent in nature as RNA molecules have diverse regulatory roles through a variety of interactions with other nucleic acids, proteins, and small molecules, some of which have been previously employed in whole-cell synthetic biology efforts (Isaacs *et al*, 2006). Furthermore, by eliminating protein regulatory roles altogether, this cell-free approach provides a key simplification by avoiding the difficulties associated with tuning gene circuits by controlling protein–DNA or protein–RNA interactions. This simplification, however, does not come at the cost of loss of functional completeness as arbitrary logic circuits and abstract neural network computations can be implemented using transcriptional circuits.

Although simpler for design, transcription-only circuits are fundamentally different from the natural genetic circuits that are being emulated. For example, translation, protein folding, and protein maturation all take time, and these delays between the initiation of gene expression and the accumulation of product may have functional consequences, especially for circuits that oscillate or involve feedback. Furthermore, translation is a 'bursty' process where multiple proteins are produced following each transcriptional event. As a result, the noise in the regulatory protein population is controlled by translation and is usually significantly higher than the Poisson noise produced by transcription alone (Thattai and van Oudenaarden, 2001). Whether such differences impact a rewriting effort or not depends on the role these effects play in the function of the emulated natural gene circuit. If needed for gene circuit function, noise, delays, or other translational effects would have to be included through the addition of other circuit elements.

Dealing with this extended list of details significantly complicates a gene circuit design effort. For example, a balance between regulatory molecule synthesis and degradation or dilution is required to achieve dynamic behavior and to reach steady-state molecular concentrations in any system. In whole-cell synthetic biology, dilution and decay come as part of the cell's degradation pathways or through growth, and often are given little thought compared to the construction of elements that control synthesis. However, Kim *et al* found that controlling degradation was more challenging than controlling synthesis. Systems containing just a few ribonucleases led to incomplete RNA degradation, build-up of inactive, partially degraded RNA, saturation of ribonucleases activity, alteration or loss of gene circuit function, or unequal degradation rates for different regulatory RNAs. Yet, it is the struggle to maintain gene circuit function in the absence of these cellular resources that is the very essence of rewriting as a discovery tool.

Unfortunately, dealing with the myriad of details in gene circuit operation and support is a double-edged sword. The cell-free environment focuses attention on system components that may be neglected in the whole-cell environment; yet it is a daunting task to recreate all housekeeping functions required for even relatively simple systems of synthetic gene circuits. As a result, cell-free and whole-cell synthetic biology are more appropriately thought of as complements rather than competitors, where each provides a different angle from which to view bottom-up rewriting of genetic systems. Indeed, a many pronged approach from nanofabricated cell mimics (Fletcher *et al*, 2004) to engineered minimal cells (Forster and Church, 2006) may be required before we reach the Feynman criteria for understanding the organization of natural genetic systems.

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