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Review

Synthetic biosensing systems

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ABSTRACT

An essential feature of synthetic biology devices is the conversion of signals from the exterior of the cell into specific cellular events such as the synthesis of a fluorescent protein. In the first synthetic gene circuits, signal transduction was accomplished via inducible or repressible transcription factors. Today, these rather simple transcription networks are the basis for the construction of more sophisticated devices that, for instance, couple artificial gene circuits with cellular pathways to create a biosensing moiety. In the future, completely artificial signaling pathways will give us the possibility to control cellular processes in a direct, precise and reliable way. At present, numerous pathway components such as receptors, adapters, scaffolds and their interactions with ligands and other signaling proteins have been already characterized and, in some cases, reengineered. In addition, important results have been obtained by rewiring pathways and building more complex gene networks—such as "cell phones" and ecosystems—based on synthetically induced cell–cell communication mechanisms. Furthermore, RNA-interference and light-dependent control of transcription factors have become new instruments to integrate different signals and better regulate protein synthesis. Overall, synthetic biology of sensing systems appears to be in continuous evolution. Nevertheless, rapid improvements on the available DNA-recombinant technology are essential to achieve, within few years, a full engineering of cell transduction pathways.

Contents

Abbreviations: RBS, ribosome binding site; GPCR, G protein coupled receptor; RTK, receptor tyrosine kinase; MAPK, mitogen-activated protein kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein; PoPS, polymerase per second; RiPS, ribosome per second; TetR, tetracycline repressor; aTC, anhydrotetracyclin; LacI, lactose repressor; IPTG, isopropyl β-D-1-thiogalactopyranoside; AraC, arabinose activator; AHL, N-acyl homoserine lactone; AI-1, N-3-oxohexanoyl-L-homoserine lactone; LuxR, luciferase activator; PIT, pristamycin-dependent transactivator; tTA, tetracycline-dependent transactivator; LOV, light-oxygen-voltage; RASSLs, receptors activated solely by synthetic ligands; SH2/3, Src (Sarcoma) homology 2/3; PTB, phosphotyrosine binding; Grb2, growth factor receptor-bound protein 2; FADD, FAS-associated death domain; Ste5/11, stellate5/11; PBS2, phosphate buffered saline 2; PDZ, post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), Zonula occludens-1 protein (zo-1); N-WASP, neuronal Wiskott-Aldrich syndrome protein; miRNA, micro RNA; siRNA, small interfering RNA.

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1. Introduction

Synthetic biology is a rather new discipline—commonly referred to as "life engineering"—that aims to design and construct new, biological systems characterized by specific and fully predictable outputs. Synthetically reengineered cells might target several important tasks from disease treatment (Ro et al., 2006) to biofuel production (Savage et al., 2008) and hazardous waste recognition and removal (de las Heras et al., 2008). Initial attempts to build synthetic circuits were mainly proof of principle studies based on the mechanisms that regulate DNA transcription. The first striking results were obtained a decade ago: the "Repressilator" (Elowitz and Leibler, 2000) and the "Toggle Switch" (Gardner et al., 2000). The Repressilator is a ring oscillating system made of three genes each synthesizing a different repressor. They are arranged in such a way that the first gene inhibits the second, the second represses the third, and the third closes the cycle by acting on the first one. The output of the circuit, a reporter protein coupled with one of the three genes, shows oscillations that can persist for hours. The "Toggle Switch" is slightly simpler because it contains only two mutually repressing genes. As a main feature, the circuit shows two stable steady states (bistability) where just one of the two genes is expressed. By deactivating the repressor with the corresponding inducer molecules, the system can be switched from one to the other stable state.

Nature equipped cells with sensing systems that allow for constant monitoring of the surrounding environment. These cellular entities can be either simple transcriptional networks or more elaborate signaling pathways. However, the task they perform is identical: recognizing external signals and transducing them to the proper cell compartment via a series of chemical reactions that demand high regulation. According to the main point of signal intervention, cellular sensing systems can be divided into three classes, namely: transcriptional, translational (or post-transcriptional), and post-translational.

Transcriptional sensing systems are gene networks where signals control promoter activities by binding to and modifying the structures of transcriptional regulator proteins with direct access to the promoter sequences. The two classic synthetic biology examples mentioned above have in common the usage of two chemically controllable transcriptional regulators: TetR and LacI. Despite the possibility of engineering new, synthetic transcription factors (Ajo-Franklin et al., 2007), the majority of the gene circuits constructed so far employs only a handful of natural repressor and activator proteins since they can be easily manipulated and directly controlled with chemicals to obtain synchronization and titration of their respective activities (see Fig. 1 for a representation of the "Toggle Switch").

Translational sensing systems exert their control by modifying the availability, the localization, the structure or the stability of mRNA molecules. Here, signals regulate protein synthesis directly by binding the mRNA at riboswitches and ribozymes (Serganov and Patel, 2007). They are RNA structures made of a single (Winkler et al., 2002; Winkler and Breaker, 2005) or a tandem (Sudarsan et al., 2006) aptamer, where the signals bind, and an actuator, which undergoes either conformational changes (riboswitches) or splicing (ribozymes) as a consequence of the signal's arrival. Their intrinsic on/off behavior makes them particularly suitable for a biological implementation of Boolean (digital) gates (Win et al., 2009). These devices work with binary (0/1) values and convert several inputs into a single output after performing a logic operation (e.g. AND: logic multiplication and OR: logic addition). In biology, 0 and 1 are translated into low and high concentration, respectively. RNA-based gates use chemicals such as thiamine and theofilline as inputs and return, as an output, the translation of a (fluorescent) protein. As an example, a simple NOT gate built on a tandem riboswitch is shown in Fig. 2.

Finally, post-translational sensing systems are made of an ensemble of proteins that, upon the arrival of a stimulus, perform a specific function such as activating or repressing the expression of some genes. Examples of this kind of systems are given by endogenous signaling pathways. Here, an extra-cellular signal is "captured" by a membrane protein (receptor) and then transmitted to a cellular component such as the nucleus or the flagellar motors—as observed in bacterial chemotaxis (Koshland, 1979)—through a cascade of reversible chemical interactions (cycles) catalyzed by enzymes. A synthetic implementation of a post-translational sensor was realized in yeast (Bashor et al., 2008). By engineering positive and negative feedback loops onto the scaffold protein of the yeast mating pathway, both qualitative and quantitative behavior of the system were changed in a predictable manner (see Fig. 3 for the scaffold configuration that mimics an ultrasensitive switch.).

In the following, we illustrate the synthetic biosensors assembled thus far on DNA/RNA, and signaling pathways. The former were, initially, small circuits—controlled by external cues—that, successively, became more complex and were based mainly on different cell–cell communication processes. The latter are still in their early age but encouraging results clearly indicate their suitability for the construction of complex networks with novel functionalities. In conclusion, we will trace some possible, future directions of synthetic biology.

2. Transcriptional and translational biosensors

2.1. Exogenous control—prokaryotic cells

Some transcription factors protein may be regarded as sensing devices since they are either activated or inactivated after binding an environmental signal (e.g. a chemical). This simple docking process finds diverse applications in nature such as the *E. coli* lac operon (Mueller-Hill, 1996) and the lysis/lysogeny control in phage lambda (Ptashne, 2004). Most of the synthetic gene circuits that were engineered in *E. coli* make use of only transcription factors together with their corresponding signals. For instance, the system TetR-aTC (Hillen and Berens, 1994) was exploited in multi-step cascade network (Hooshangi et al., 2005) to either switch on or off the production of a fluorescent protein depending on number of steps in the cascade. Furthermore, a bacterial tunable oscillator (Stricker et al., 2008) was recently implemented by coupling the LacI-IPTG system with an activator (AraC) induced by arabinose molecules. Here, the two chemicals were used to alter the period of the oscillations.

As an important effect of transcription regulation, several synthetic and wild type promoters reproduce Boolean gates (Bintu et al., 2005; Silva-Rocha and de Lorenzo, 2008). Therefore, they can be exploited to build biological digital circuits that represent a means to develop new, efficient sensors for drug screening. In fact, thanks to the unequivocal input/output relation expressed into a truth table, synthetic gene digital circuits appear to be the most promising solution to properly integrate different input signals in a single readout.

Remarkably, transcription factors can be regulated by inputs different from chemicals. Lou et al. (2010), for instance, implemented in *E. coli* a "push-on push-off" digital switch by controlling transcription with light. The output of this circuit is described as discrete (either 1 or 0) and can be switched between these two values by means of a luminous input signal. In another relevant application, *E. coli* cells were engineered to express the invasin gene (from *Yersinia pseudotuberculosis*) in response to a well-defined environ-