Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Engineering mammalian cells for disease diagnosis and treatment

Ana P Teixeira[1] and Martin Fussenegger[1,2]

Synthetic biology aims to repurpose cells to sense a wide range of input signals and respond by conditionally expressing user-defined output genes. In this review, we highlight the latest developments in synthetic-biology-inspired *in vitro* and *in vivo* diagnostics and therapeutics employing engineered mammalian cells. Recent work has led to the creation of modular synthetic receptors with adaptable ligand-binding domains whose recognition specificity can be easily tailored to target various diseases. Engineered cells can now sense a great variety of soluble and surface-bound antigens with unprecedented selectivity and sensitivity, and implanted designer cells equipped with appropriate response modules can successfully treat diabetes, cancer and autoimmune diseases in animal models. Recently, the first immunotherapies using chimeric-antigen receptor (CAR)-T cells were approved to treat lymphoma patients. As this milestone paves the way for translating state-of-the-art synthetic biology approaches into clinical benefit, we also discuss the challenges facing engineered cell therapies.

Addresses
[1] Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland
[2] University of Basel, Faculty of Science, Basel, Switzerland

Corresponding author:
Fussenegger, Martin (fussenegger@bsse.ethz.ch)

Current Opinion in Biotechnology 2018, **55**:87–94

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Saulius Klimasauskas** and **Linas Mazutis**

https://doi.org/10.1016/j.copbio.2018.08.008

Introduction

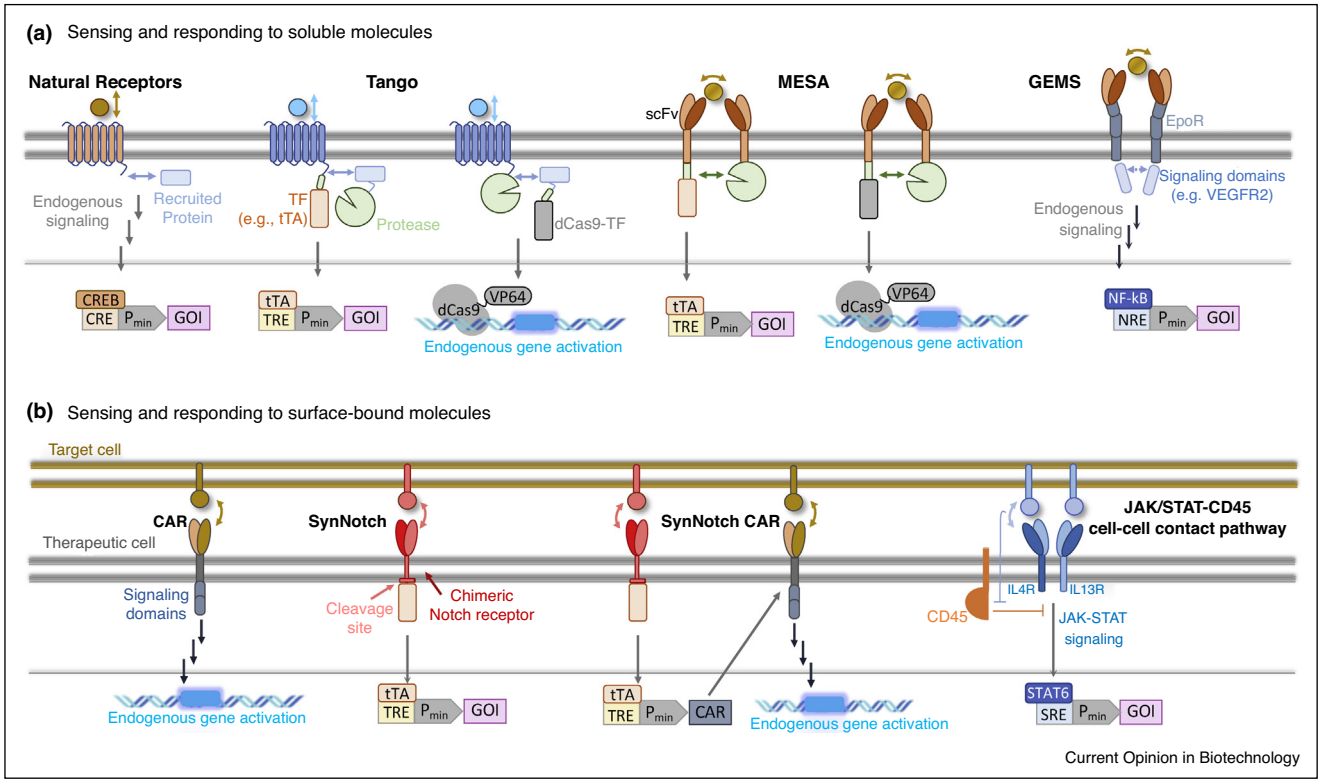
Living cells host a vast array of evolved sensors, actuators and regulators that serve to maintain intracellular homeostasis. Among them, transmembrane receptors continuously monitor the extracellular milieu, and transduce specific extracellular signals into intracellular responses. Binding of ligands to their cognate receptors triggers intracellular signaling cascades that ultimately induce transcriptional changes. Synthetic biologists have been leveraging the sensory and biosynthetic capabilities of cells to build gene circuits repurposed for a range of different applications, including cell-based diagnostic and therapeutic devices. Both mammalian and bacterial cells have been used as biological chassis to create devices capable of interfacing directly with human disease signals, and computing and executing easily measurable or appropriate therapeutic outputs. As the body of work on bacterial cells was comprehensively overviewed very recently [1], we will restrict our discussion to mammalian cells.

Most synthetic devices are based on networks regulated by transcription factors (TFs). Early mammalian circuits were largely assembled with TFs from bacteria or yeast (*e.g.* TetR or GAL4) fused to transcriptional activation or repression domains (*e.g.* VP16 or KRAB). However, in recent years endogenous TFs, such as nuclear factor of activated T cells (NFAT), cAMP response element binding (CREB), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and signal transducer and activator of transcription (STAT), which are activated by upstream native or chimeric receptors and their signaling cascades, have been widely adopted for conditional expression of user-defined transgenes. Furthermore, the development of technologies enabling programmable DNA-binding domains, such as the clustered regularly interspaced short palindromic repeats (Crispr)/Cas system [2], has stimulated the development of synthetic circuits targeting endogenous gene regulation. Here, we review the available repertoire of tools for building mammalian cells that sense soluble or cell-surface-bound molecules and respond in customized ways, and we discuss how these cells are being applied to meet the growing demand for better diagnostic and therapeutic devices.

Sensing-response circuits for soluble molecules

The simplest way to create devices that sense and respond to pathological levels of soluble cues is to capitalize on native ligand–receptor interactions and to rewire the corresponding signaling cascades to execute user-defined responses (Figure 1a). This strategy has been adopted to engineer mammalian cells to sense allergy-associated histamine levels, or high levels of bile acids associated with impaired liver function, by overexpressing the histamine HRH2 receptor or the bile-acid TGR5 receptor, respectively [3,4]. Both of these GPCRs use the cAMP signaling pathway, which was rewired to activate desired transcriptional outputs. Moreover, ion

Figure 1



Natural and synthetic receptors have been used to repurpose mammalian cells to sense specific **(a)** soluble or **(b)** cell-surface-bound molecules and respond with user-defined transcriptional outputs (exogenous or endogenous genes). See main text for description of the different systems. CREB — cAMP response element binding; CRE — CREB response element; EpoR — erythropoietin receptor; GOI — gene of interest; NF-kB — nuclear factor kappa-light-chain-enhancer of activated B cells; NRE — NF-kB response element; P_{min} — minimal promoter, most often derived from hCMV promoter; STAT6 — signal transducer and activator of transcription 6; SRE — STAT6 response element; tTA — tetracycline controlled transactivator; TRE — tetracycline response elements; TF — transcription factor.

channels have also been explored as mediators to provide cells with novel sensory functions. For instance, HEK cells overexpressing a voltage-gated calcium channel could trigger native calcium signaling and activation of NFAT transcription factors in response to high glucose levels; using NFAT-responsive promoters, it was possible to obtain hyperglycemia-induced transgene expression [5•].

Another class of synthetic sensor-and-response circuits relies on orthogonal, protease-based signal transduction modules. The so-called Tango receptors consist of native or evolved receptors fused intracellularly with synthetic TFs (*e.g.* tTA) through a flexible linker containing a protease cleavage site [6]. Binding of a ligand to its receptor recruits a signaling protein fused to the target protease, which cuts and releases the TF from the membrane, allowing it to enter the nucleus and activate expression of user-defined genes (Figure 1a). Different GPCRs, receptor tyrosine kinases (RTKs) and steroid hormone receptors have been redirected to activate transgene expression using the Tango approach [6].

Despite the available options, many molecules that would be valuable cues for diagnostic or therapeutic purposes are not recognized by any known natural receptor. To expand the repertoire of molecules that can be sensed by engineered cells, synthetic receptors have been fused extracellularly with antibody single-chain variable fragments (scFvs) selected from phage display libraries to target small-molecule-based or protein-based antigens. This approach has been adopted in the modular extracellular sensor architecture (MESA) platform using scFvs against vascular epidermal growth factor [7,8]. As Tango-like receptors, MESA receptors are based on sequestering TFs at the plasma membrane, followed by proteolytic release upon receptor activation by soluble ligands (Figure 1a). But, unlike the Tango receptors, MESA receptors rely on ligand-induced dimerization of receptor chains: one chain is fused to the TF by a protease cleavage site and the other chain to the appropriate protease. Schwarz *et al.* [8] also demonstrated that MESA receptors can be adapted to regulate endogenous gene expression by using a catalytically dead Cas9 protein (dCas9) fused to a transcriptional activation domain