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Reprogramming cellular functions with engineered membrane proteins

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Taking inspiration from Nature, synthetic biology utilizes and modifies biological components to expand the range of biological functions for engineering new practical devices and therapeutics. While early breakthroughs mainly concerned the design of gene circuits, recent efforts have focused on engineering signaling pathways to reprogram cellular functions. Since signal transduction across cell membranes initiates and controls intracellular signaling, membrane receptors have been targeted by diverse protein engineering approaches despite limited mechanistic understanding of their function. The modular architecture of several receptor families has enabled the empirical construction of chimeric receptors combining domains from distinct native receptors which have found successful immunotherapeutic applications. Meanwhile, progress in membrane protein structure determination, computational modeling and rational design promise to foster the engineering of a broader range of membrane receptor functions. Marrying empirical and rational membrane protein engineering approaches should enable the reprogramming of cells with widely diverse fine-tuned functions.

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Introduction

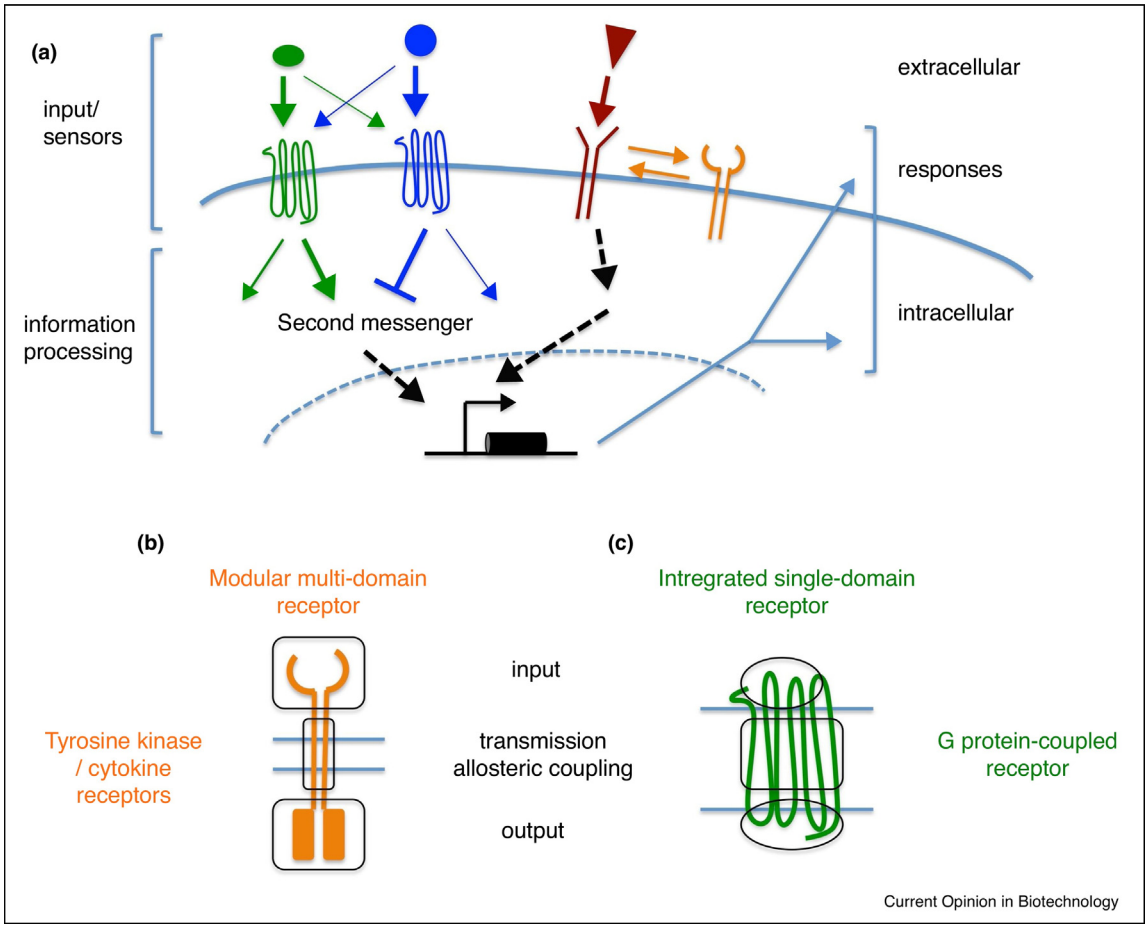
The field of synthetic biology utilizes and engineers biological systems to increase the spectrum of cellular functions for basic and translational research questions and therapeutic applications. Cell engineering technologies have made tremendous progress in recent years and are becoming central for synthetic biology applications. For example, engineered cells with customized signaling responses to disease-associated molecules provide promising and powerful new therapeutic agents for cancer immunotherapy, regenerative medicine and autoimmune disorders [1,2].

Early synthetic biology efforts have largely focused on engineering gene circuits in bacteria [3]. Since cellular functions are controlled by complex networks of signaling proteins, research efforts have expanded to the engineering of signaling pathways (Figure 1). One common universal aspect of cell signaling is the central role played by communication across cellular membranes in initiating and controlling the outcome of intracellular signaling cascades. Faithful communications across membranes is achieved by complex extracellular ligand-membrane receptor-intracellular effector systems.

Membrane receptors represent a large fraction of membrane proteins which constitute around 30% of all genome-encoded proteins. Upon sensing extracellular stimuli, membrane receptors transmit the extracellular signals across the cell membrane through allosteric molecular mechanisms involving long-range cooperative structural and dynamics changes that are difficult to study and poorly understood [4]. Consequently, the signaling properties of membrane receptors have remained challenging to engineer using rational protein engineering approaches. Recent progress in structure-based and rational membrane protein design techniques promise to expand the range of receptors and functions that can be redesigned [5]. Meanwhile, empirical synthetic biology approaches have exploited the modularity of few membrane protein families for creating synthetic chimeric receptors to redirect cellular signaling, and some have been successfully translated to clinical application in engineered cell therapies [6].

In this review, we will first discuss empirical approaches taking advantage of membrane receptor modularity for engineering chimeric receptors and highlight recent success and promises for the near future [7••]. We will then discuss emerging strategies for engineering intrinsic properties of membrane receptors enabling the engineering of a broader range of fine-tuned protein functions. While seminal and pioneering studies have targeted prokaryotic signaling systems [8,9], we will focus our discussions on

Figure 1



Membrane receptor systems control intracellular signaling. (a) Schematic depiction of cellular signaling pathways triggered at the cell surface by extracellular stimuli. Upon sensing by membrane receptors (input/sensors), extracellular signals are transmitted inside the cell, processed into distinct signaling cascades (information processing) leading ultimately to regulated gene expression and selective cell responses. **(b and c)** two major classes of membrane receptors encode similar functional elements into distinct protein topologies (modular (b) or integrated (c)).

eukaryotic systems. We also recognize that the field of optogenetics has been growing rapidly with the engineering of photosensitive membrane protein chimeras providing spatiotemporal control of signaling function. These innovations have been covered in several recent reviews [10,11] and will not be discussed here.

Manipulating receptor signaling through modular design

Synthetic biology approaches have initially targeted single pass membrane receptor families, such as tyrosine kinase and cytokine receptors, despite poor mechanistic understanding of their signal transduction. The modularity of these receptor folds (Figure 1b) has guided empirical trial and error approaches that incorporate protein domains into chimeric receptors displaying intended novel functions. Table 1 gives an overview of the different types of chimeric receptors designed so far, the type of reprogramming, and which cellular behavior was produced.

Empirical modular design approaches have led to the development of tumor-targeted chimeric antigen receptors (CARs) (Figure 2a), aiming at efficiently redirecting T cell antigen-specificity to tumor cells for immunotherapy of cancer [1,6,12••]. An antigen-recognition domain (usually an antibody-derived single chain variable fragment, scFv) is linked to a transmembrane and signaling domain, comprising various co-stimulatory receptor endodomains and CD3ζ for T cell activation. Parameters critical for functional CAR design include the choice of scFv for a given target, hinge origin and linker length (dependent on the scFv binding site of the target antigen), propensity for autonomous CAR signaling, choice of TM domain, the type of co-stimulatory endo-domain(s), and the method and culture conditions used to introduce the transgene and produce CAR T cells (CARTs). Since