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The best of both worlds: reaping the benefits from mammalian and bacterial therapeutic circuits

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Synthetic biology has revolutionized the field of biology in the last two decades. By taking apart natural systems and recombining engineered parts in novel constellations, it has not only unlocked a staggering variety of biological control mechanisms but it has also created a panoply of biomedical achievements, such as innovative diagnostics and therapies. The most common mode of action in the field of synthetic biology is mediated by synthetic gene circuits assembled in a systematic and rational manner. This review covers the most recent therapeutic gene circuits implemented in mammalian and bacterial cells designed for the diagnosis and therapy of an extensive array of diseases. Highlighting new tools for therapeutic gene circuits, we describe a future that holds a plethora of potentialities for the medicine of tomorrow.

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Introduction

Synthetic biology, the science of designing novel, biological systems with the help of engineering [1], is currently at a crossroads. Being on the cusp of the transition from proof-of-concept experiments to 'real-world' applications [2], synthetic biology and its genetic circuits now need more precision and robustness than ever [3,4]. Starting from simple modules, such as toggle switches [5] and oscillators [6], the basic components created by researchers over the years have been recombined extensively to yield complex and innovative genetic circuits that can perform intricate logic operations [7].

One of the main fields of application relevant for synthetic biology is biomedical research [8], including 'smart' diagnostics and therapies in the form of complex genetic circuits [9–11]. These designed therapies have not only been developed in mammalian cells but also in bacteria, such as the biological model organism *Escherichia coli* (*E. coli*) [12,13], which are much simpler and therefore easier to engineer. Utilizing the full potential of synthetic biology, exciting proof-of-concept gene circuits have been assembled in the past, including RNA interference (RNAi) by cancer-invading bacteria [14] and mammalian cells that can process several inputs in order to identify and kill specific cancer cells [15]. This review focuses on the latest, state-of-the-art synthetic gene circuits and will first discuss the new and promising field of *ex vivo* diagnostics, followed by an in-depth discussion of circuits designed as therapeutics for human diseases in both mammalian as well as in bacterial cells and concluding with an outlook into the future of the synergy between synthetic biology and medicine.

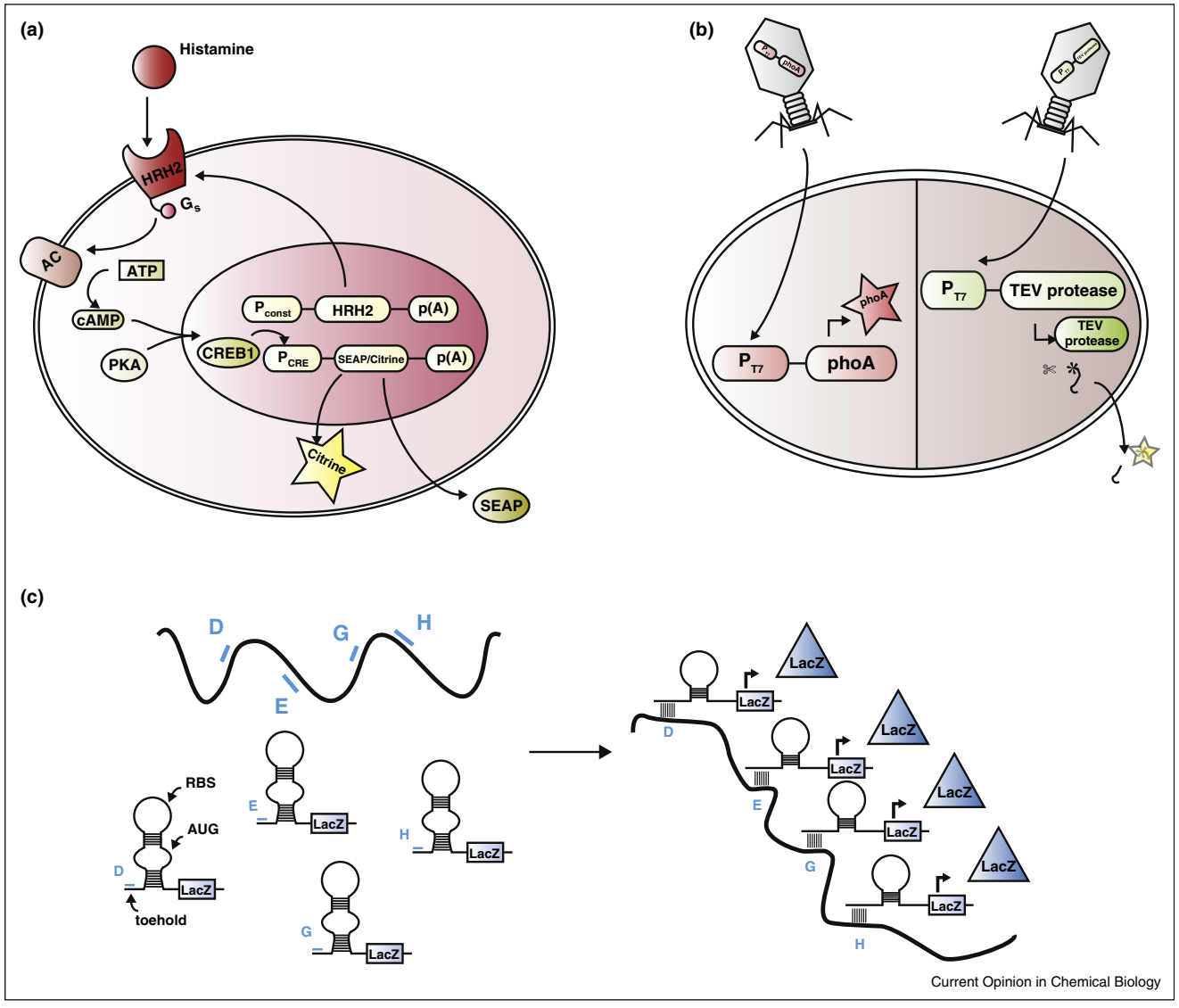
The emerging field of *ex vivo* diagnostics

One of the major issues of the transitions from animal models to clinical trials is the limited probability of success [16]. As Seok *et al.* showed, mice and humans differ substantially in their response to inflammatory stress, portending grave differences in the effect of genetic circuits designed to alleviate these stresses [17]. One way to address this conundrum is to replace animal models with *ex vivo* diagnostics, which have been shown to exhibit higher sensitivity in release assays of IFN-γ [18].

One area that suffers from this limited correlation between the results in model systems and those in the human body is the *in vitro* diagnosis of allergies based on the measurement of free immunoglobulin E (IgE) antibodies [19,20]. Here, Ausländer *et al.* recently reported the design and implementation of an *ex vivo* allergy profiler [21••]. This device detects the characteristic increase in histamine secreted by basophil granulocytes upon addition of the respective allergen (Figure 1a). By rewiring a reporter gene to a histamine-responsive promoter, the authors demonstrated the reliable and sensitive functionality of their system, which was a significant improvement on the above mentioned IgE quantification and the classic, though impractical, skin-prick test [22].

Upcoming bacteriophage-based diagnostics tools are a valuable addition to the existing toolbox, mainly because of their ability to rapidly and precisely identify pathogenic bacteria. Two recent studies by Alcaine *et al.* elucidated this trend very clearly. In the first study, engineered T7

Figure 1



Gene circuits for *ex vivo* diagnostics. **(a)** Gene circuit for the *ex vivo* profiling of allergies [21]. Rising histamine levels activate histamine receptor H2 (HRH2), in turn activating adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Binding to protein kinase A (PKA), an increase in cAMP causes the phosphorylation of cAMP responsive elements binding protein 1 (CREB1). The binding of CREB1 to its respective DNA elements results in the production of the reporter proteins SEAP and Citrine, enabling quantification of the allergic response. **(b)** Bacteriophage-based identification of pathogenic bacteria [23,24]. Bacteriophages carrying the plasmids encoding either alkaline phosphatase (phoA) or tobacco etch virus (TEV) protease, which are able to specifically infect one type of bacteria, can be used for the quantitative detection of pathogenic bacteria. After infection and expression of the respective protein, AP can be assayed by the addition and conversion of substrates such as para-Nitrophenylphosphate (pNPP), TEV protease is assayed by cleavage reactions that produce fluorescent peptides. **(c)** Paper-based gene circuit for the identification of Ebola virus strains [25]. Sensor regions on the viral mRNA are complementary to the toehold region of the diagnostic RNA toehold switches. By recruiting these sensor regions to the toehold region, additional base pairing is initiated in the region of the stem loop. Leading to a major structural rearrangement by freeing up the ribosome binding site (RBS) and start codon (AUG), the bound toehold switches express the reporter gene LacZ, which can be quantified using a colorimetric reaction. This allows for differentiation between Sudan and Zaire strains of the Ebola virus by choosing appropriate sensor regions.

bacteriophages transported and overexpressed the common reporter gene alkaline phosphatase in infected *E. coli*, allowing the determination of the resistance of a given *E. coli* to the antibiotic ampicillin [23]. The second study, which also employed engineered T7 bacteriophages, transported and overexpressed tobacco etch virus (TEV) protease in targeted *E. coli* upon bacteriophage infection [24]. This ectopically expressed protease