ACS SyntheticBiology

In This Issue

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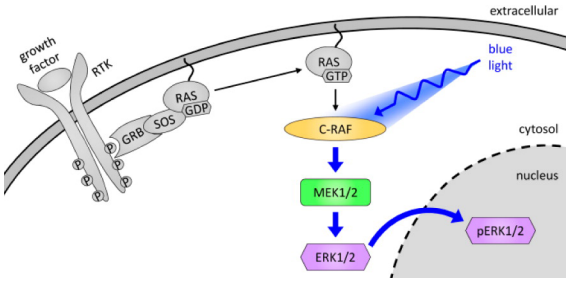
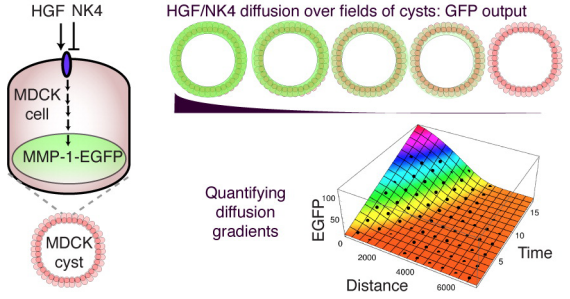
OPTOGENETIC CONTROL OF PROTEIN KINASE ACTIVITY



Signaling networks are the basis of all biological processes. Protein kinases are one of the major mediators of signals from the cell surface to the nucleus to regulate gene expression. Using the protein kinase C-RAF as prototype, Wend *et al.* (DOI: 10.1021/sb400090s) developed a tool to bring a kinase under light-control in mammalian cells.

The blue light sensitive photoreceptor from *Arabidopsis thaliana* CRY2 fused to wild-type C-RAF allows light-dependent dimerization of the fusion protein leading to stimulation of the C-RAF kinase activity. Illumination induces rapid, reversible and tunable activation of C-RAF. Thus, downstream signaling including feedback mechanisms can be studied by this tool uncoupled from upstream inputs such as mitogen-activated receptors. Mutants of the isoform B-RAF have a high impact on tumorigenesis. Light-induced hetero-dimerization of C-RAF and kinase-dead B-RAF mimics the paradoxical activation of C-RAF and allows detailed studies of this mechanism. Based on this study other light-regulated protein kinases can be engineered and will be a valuable tool for research in synthetic biology.

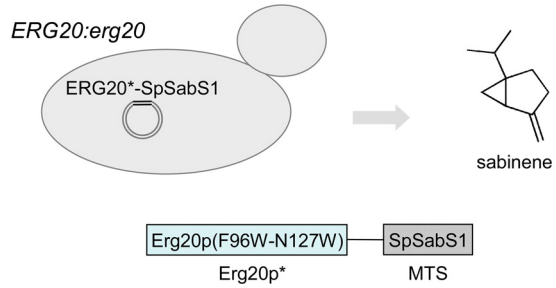
A GENETICALLY ENCODED SENDER–RECEIVER SYSTEM



Engineering spatial patterns is a major goal in synthetic biology. While most of the literature to date focus on gene networks in bacteria, the literature on mammalian synthetic biology remains sparse. Now, Carvalho and Menendez *et al.* (DOI: 10.1021/sb400053b) provide a toolkit for engineering cell–cell communication networks in 3D mammalian cell culture.

The authors characterize components for achieving inducible cellular expression, secretion, diffusion, and either activation or repression in nearby receiver cells. Each component was characterized individually, resulting in a reliable system that gives very reproducible behavior. Additionally, the system described here uses only genetically encoded components thus allowing for the ability to engineer more complicated multicellular feedback networks.

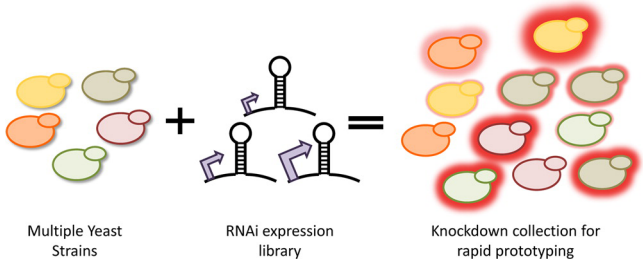
ENGINEERING MONOTERPENE PRODUCTION IN YEAST



Terpenes have numerous applications, especially in pharmaceuticals, fragrances, or biofuels. With increasing interest in producing terpenes sustainably and economically, there has been significant progress in recent years in developing methods for their production in microorganisms. In *S. cerevisiae*, production of the 10-carbon monoterpenes has so far proven to be significantly less efficient than production of larger terpenes. Here, Ignea *et al.* (DOI: 10.1021/sb400115e) identify the sequential mechanism of farnesyl diphosphate synthesis by Erg20p to be a major limitation in monoterpene productivity.

The authors engineered Erg20p into a dominant negative geranyl diphosphate synthase (i.e., an enzyme that synthesizes geranyl instead of farnesyl diphosphate) and at the same time inhibits farnesyl diphosphate synthesis by the wild-type endogenous Erg20p. Using this synthetic part, they demonstrate a higher than 300-fold overall improvement in productivity. The design developed here can be applied to the production of any monoterpene and is compatible with most yeast chassis.

OPTIMIZATION OF A YEAST RNAi SYSTEM FOR CONTROLLING GENE EXPRESSION



While the ability to reduce endogenous gene expression is fundamental to metabolic engineering, current methods for gene knockdown, such as genome editing, remain laborious and slow, especially in yeast. Now, Crook *et al.* (DOI: 10.1021/sb4001432) describe the optimization and utility of a synthetic RNA interference system in *S. cerevisiae*.

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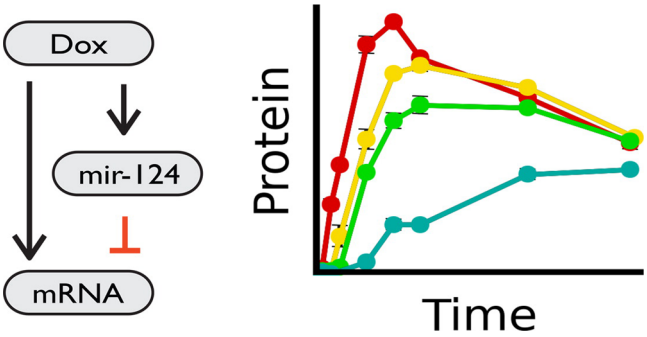
The authors demonstrate several design cycles to optimize the construction of hairpin RNA expression cassettes in yeast and demonstrate how such an approach can enable rapid prototyping of knockdown strategies and accelerate the design-build-test cycle. By simultaneously testing the impact of gene knockdowns in multiple strains, they further show that rapid strain prototyping is possible in yeast through the use of a synthetic RNAi system. This work allows for rapid metabolic engineering in yeast using RNAi, which enables wider exploration of knockout targets, more finely tuned control of knockdown level, and greater flexibility in strain evaluation.

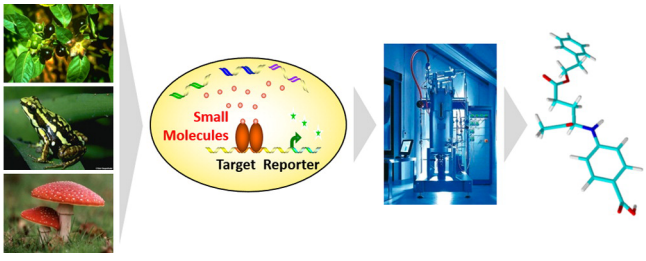
MICRORNA-BASED SINGLE-GENE CIRCUITS BUFFER PROTEIN SYNTHESIS RATES AGAINST PERTURBATIONS



Achieving precise control of mammalian gene expression is critical for cell and developmental biology, and biomedical applications. However, there are currently no modular, feasible approaches for meeting this goal. In this study, Strovas and Rosenberg *et al.* (DOI: 10.1021/sb4001867) introduce a general microRNA-based mechanism for buffering protein synthesis rates against transcriptional noise and environmental changes.

The authors engineered a minimal autoregulatory gene circuit consisting of an intronic miRNA that targets its host transcript and stably integrated this construct into the genome of mammalian cells. This genomic integration enabled the study of how this system responds dynamically to sustained perturbations and allowed the authors to investigate buffering of gene expression at the single gene copy level. The results provide fundamental insights into the dynamic behavior of an important biological network motif.

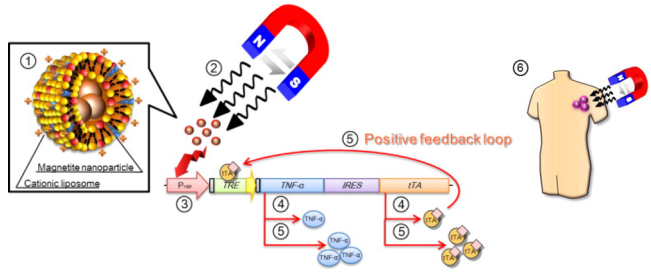
GENERATION OF NOVEL CHEMICAL STRUCTURES AS SCAFFOLDS FOR DRUG DISCOVERY



Compounds produced by synthetic biology possess favorable properties that qualify them as good starting points for lead optimization. Here, Klein et. al (DOI: 10.1021/sb400177x) describe the production of small molecules using the baker's yeast, *S. cerevisiae*.

Genetic material was introduced into the yeast and expressed from artificial chromosomes, creating a multitude of new heterologous pathways. A survival assay was also incorporated into the cells. Identified active compounds were considered as prevalidated hits; 75% were previously not described elsewhere and 20% of the compounds exhibited novel and diverse scaffolds. The resulting fragment to scaffold-sized molecules also retained biological activity. The synthetic biology approach detailed here represents a completely new, complementary hit and early lead finding strategy that can be easily integrated into the existing drug discovery process.

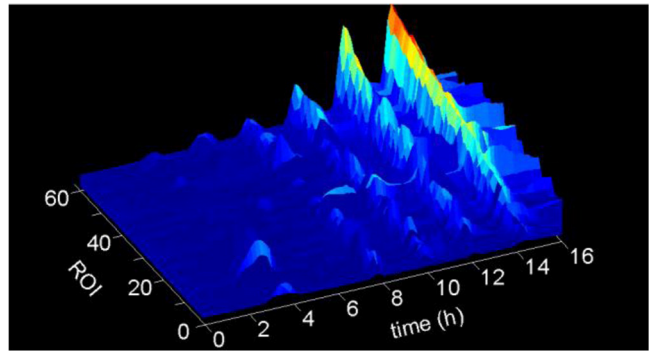
A HEAT-INDUCIBLE GENE EXPRESSION SYSTEM



Gene therapy has a wide range of applications in medicine, including cancer and some neurological disorders. Controlled gene expression is essential for gene therapy, and thus, a key focus of synthetic biology is the design of artificial gene expression systems. Here, Yamaguchi *et al.* (DOI: 10.1021/sb4000838) combine synthetic biology and nanotechnology to develop a heat-inducible gene expression system.

Magnetite nanoparticles generate heat under an alternating magnetic field (AMF). The authors transfected cells labeled with magnetite nanoparticles with a heat-inducible gene expression plasmid and saw that target gene expression was induced by AMF exposure. Using this system in a tumor xenograft model, they showed significant tumor growth inhibition. Thus, this system shows great potential for controlling gene expression in a spatiotemporal manner and may be used for remote control of cell functions using nanoparticles and magnetic fields.

USING A STOCHASTIC BISTABLE SWITCH TO COORDINATE RESPONSES OF AUTONOMOUS BACTERIA



The origin of noise, inherent to single cell behavior, can be traced to the stochasticity associated with a few copies of genes and low concentrations of protein and ligands. In this paper, Nelson *et al.* (DOI: 10.1021/sb400052f) study the mechanisms by which the response of noisy elements can be entrained for biological signal processing in a synthetic biofilm.

The authors forged a gene environment in a synthetic biofilm that leverages the positive feedback found in quorum sensing regulatory components in the *lux* operon that are used to

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coordinate cellular responses to fluctuations in the environment. They show that the memory of the system channels stochastic noise into an apparent tight coordination of the responses between quorum sensing signal receivers. The noise in the receiver diminishes with repeated exposure to both a quiet exogenous signal and to noisy transmitters on the input of a signaling cascade integrated into the same synthetic biofilm.

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