ACS SyntheticBiology

In This Issue

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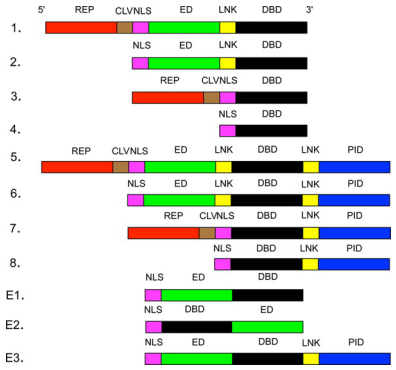
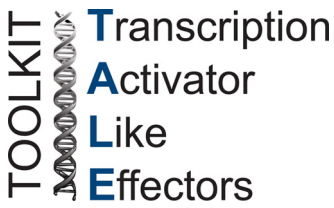
RULE-BASED DESIGN OF SYNTHETIC TRANSCRIPTION FACTORS



As the field of synthetic biology matures and biological engineers aim to construct increasingly large and complex biological systems, it will become vital that design tools are available to make the design process as simple and reliable as possible. One such tool is GenoCAD, which through the use of rules or 'grammars' guides users to construct biological systems that are 'valid' and function correctly. However, in order for GenoCAD to be useful to synthetic biologists, it must maintain an up-do-date set of components from which to build these systems. In this Technical Note, Purcell *et al.* (DOI: 10.1021/sb400134k) construct a grammar for the design of eukaryotic synthetic transcription factors (sTFs), the essential components for the construction of synthetic gene networks.

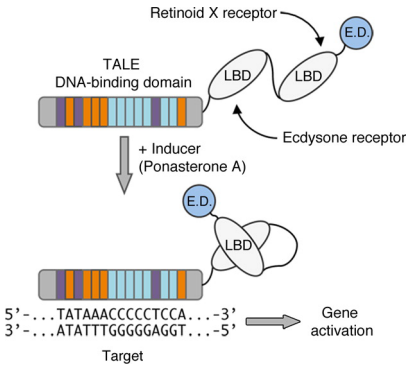
The authors base their grammar on the current literature of sTFs and their own experience. This grammar allows everyone to rapidly and reliably design sTFs, based on either Zinc Fingers, TALEs or the CRISPR/Cas system, that can act as activators or repressors, be quantified using reporters, and be made to exhibit cooperativity through homodimerization. The authors make the grammar available upon request and anticipate that it will be refined and expanded to keep pace with the latest knowledge of sTF design.

TRANSCRIPTION ACTIVATOR-LIKE EFFECTORS



Transcription activator-like effectors (TALEs) are proteins secreted by *Xanthomonas* bacteria to infect plant species by binding to specific DNA sequences and activating the expression of genes. Considering the highly modular nature of TALEs and the ease of constructing these proteins, this technology can have important implications for synthetic biology. Here, Moore, Chandrahas and Bleris (DOI: 10.1021/sb400137b) review developments in the area with a focus on modifications for custom and controllable gene regulation.

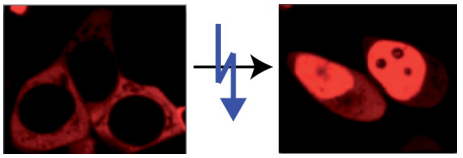
REGULATION OF ENDOGENOUS HUMAN GENE EXPRESSION



Transcription activator-like effectors (TALEs) are modular DNA binding proteins that can be rapidly designed and constructed to target any DNA sequences. Here, Mercer *et al.* (DOI: 10.1021/sb400114p) describe a new class of synthetic transcription factors that activate gene expression in response to extracellular chemical stimuli.

These inducible activators consist of customizable transcription activator-like effector (TALE) proteins combined with steroid hormone receptor ligand-binding domains. The authors demonstrate that these ligand-responsive TALE transcription factors allow for tunable and conditional control of gene activation and can be used to regulate the expression of endogenous genes in human cells. The work described here thus enables the design of advanced synthetic gene networks.

OPTOCHEMICAL TRANSLOCATION TO CONTROL PROTEIN FUNCTION



In order to understand proteins and their function/role in a cell's life, it is important to be able to control their function spatially and temporally. The best way to gain spatial and temporal control is via light. Efforts have been made to address this challenge, mainly by direct manipulation of the active site of the protein. However, such constructs are hard to adapt to other proteins. Now, Engelke *et al.* (DOI: 10.1021/sb400192a) control protein function with light via its translocation into the nucleus.

The authors achieve optogenetic control by introducing a photocaged amino acid into the nuclear localization signal. UV-illumination uncages the amino acid and releases the protein into the nucleus. This technique is easily adaptable to a wide

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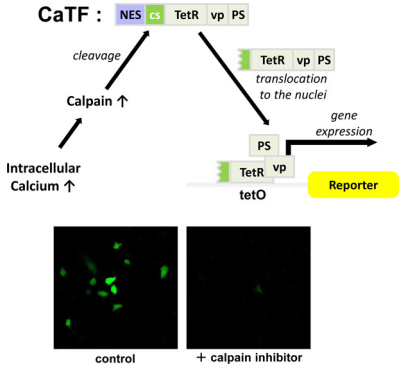
range of proteins as only the relatively small nuclear localization signal has to be added to the protein, replacing its native signal.

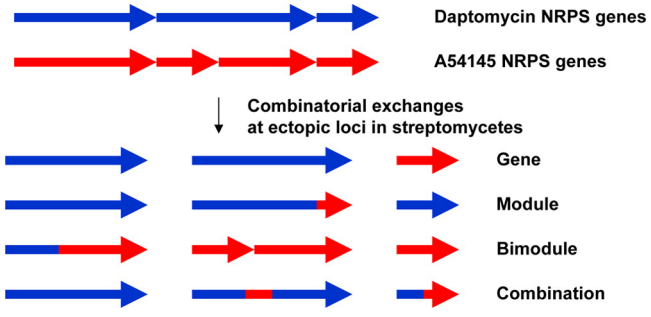
DEVELOPMENT OF AN ARTIFICIAL CALCIUM-DEPENDENT TRANSCRIPTION FACTOR



Intracellular calcium is a secondary messenger that plays a role in slew of biological processes, including transduction of survival signals, muscle contraction, and synaptic transmission. Previous work has described protein sensors for the real time imaging of intracellular calcium. However, a synthetic transcription factor that responds to intracellular calcium signals would enable the analysis of cellular events at the single-cell level. In this study, Suzuki *et al.* (DOI: 10.1021/sb500070c) develop an artificial transcription factor which induces reporter expression in response to intracellular calcium levels.

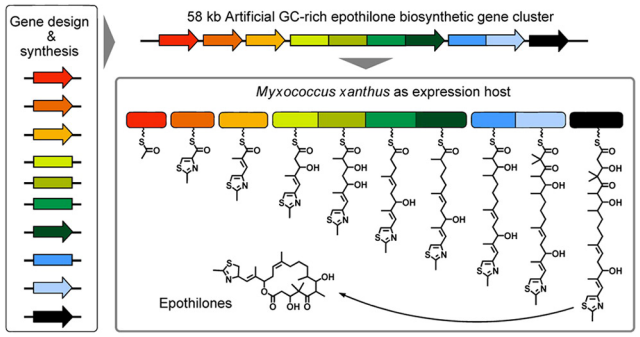
The authors developed the calcium-dependent transcription factor (CaTF), which is cleaved by calpain and then translocated to the nuclei where it induces reporter expression. The results described here suggest that the CaTF could be a useful tool in the analysis of intracellular calcium signals and as an interface between an endogenous signal and synthetic gene network.

COMBINATORIAL BIOSYNTHESIS OF CYCLIC LIPOPEPTIDE ANTIBIOTICS



One of the goals of synthetic biology is to "improve the process of genetic engineering". Nonribosomal peptide synthetases (NRPSs) are giant multienzymes that carry out sequential couplings of amino acids to generate linear or cyclic peptides. In this review, Baltz (DOI: 10.1021/sb3000673) discusses what is known thus far about the "rules" for successful recombination of NRPS functional domains (toward the development of functional peptide coupling devices) and also discusses experiments that address focused mutagenesis to quickly optimize engineered peptide assembly machines for enhanced production.

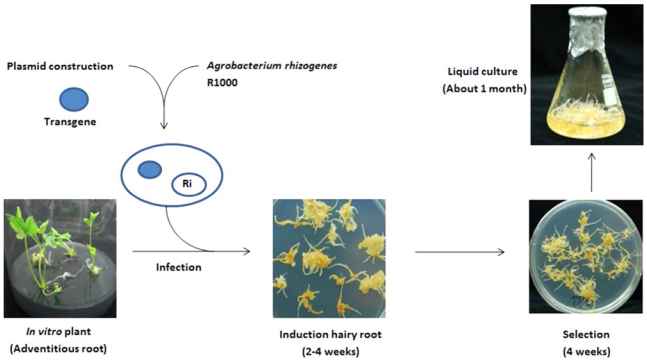
MODULAR CONSTRUCTION OF A FUNCTIONAL ARTIFICIAL EPOTHILONE POLYKETIDE PATHWAY



While natural products derived from microbes are an important source of pharmaceuticals and agrochemicals, their structural complexity makes chemical synthesis challenging. An additional challenge is the optimization of the pharmaceutical properties of these natural products, often via medicinal chemistry or biosynthetic engineering, for their desired applications. Thus, a detailed understanding of the biosynthetic process, along with genetic tools to modify the producing organism, is essential. Now, Oßwald *et al.* (DOI: 10.1021/sb300080t) describe the design, construction and heterologous expression of a complex natural product biosynthetic pathway based on synthetic DNA.

The authors redesigned and reassembled the 56 kb epothilone biosynthetic gene cluster from *Sorangium cellulosum* for expression in the high GC host *Myxococcus xanthus*. The codon composition was adapted to the codon usage of the host strain and restriction sites were engineered in order to permit pathway assembly and future interchangeability of the modular building blocks from the epothilone megasynthetase. Further, the functionality of the artificial pathway was demonstrated by successful heterologous epothilone production in *M. xanthus*. This study sets the stage for future engineering of epothilone biosynthesis and production optimization using a highly flexible assembly strategy.

ENHANCED TRITERPENE ACCUMULATION IN *PANAX GINSENG* HAIRY ROOTS



Isoprenoids are one of the largest structurally varied groups of natural products. They play vital roles in plant metabolism and are synthesized by both, the mevalonate pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. In this study, Kim *et al.* (DOI: 10.1021/sb400194g) elucidate the function of two enzymes of the mevalonate pathway in isoprenoid biosynthesis.

The genes governing the expression of the two enzymes, mevalonate-5-pyrophosphate decarboxylase (MVD) and farnesyl pyrophosphate synthase (FPS), were transformed into

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Panax ginseng hairy roots. The resulting transgenic lines all showed increased triterpene biosynthesis suggesting that metabolic engineering in *P. ginseng* was successfully achieved through *Agrobacterium rhizogenes*-mediated transformation.

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