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In This Issue

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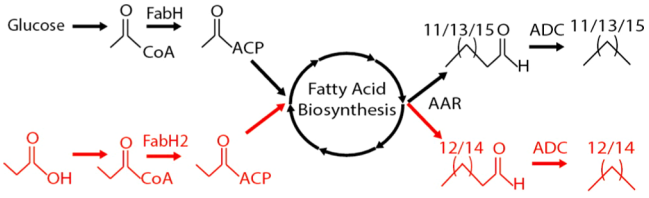
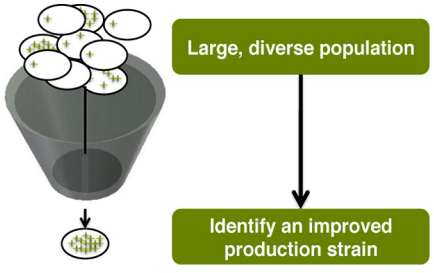
EXPANDING AN ALKANE BIOSYNTHETIC PATHWAY



Microbially produced alkanes, generated from the fatty acid biosynthetic pathway, are a new class of biofuels that closely match the chemical composition of petroleum-based fuels. A limitation of this pathway, however, is its restricted product profile. Here, Harger *et al.* (DOI: 10.1021/sb300061x) describe an expansion of this product profile by introducing a new enzyme, FabH2 from *Bacillus subtilis*, into *E. coli*.

FabH2 has a broader specificity profile for fatty acid initiation than the native FabH of *E. coli*. Thus, the authors aimed to broaden the downstream alkane production profile by modifying the upstream intermediates in fatty acid biosynthesis. The work described here is a promising step forward in the synthesis of complex biofuels.

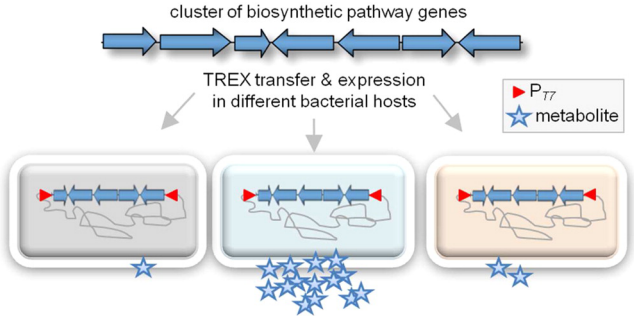
SCREENS AND SELECTIONS FOR SMALL MOLECULE BIOSYNTHESIS



The predominant challenge in the construction of *de novo* small-molecule biosynthetic pathways is rapidly shifting from the proof-of-principle demonstration of metabolic pathways to directed evolution of pathways and strains for improved yields and productivities. Strain optimization is hindered by the complexity of the microbial host; high-throughput screens or selections are necessary to address this hurdle. Here Dietrich *et al.* (DOI: 10.1021/sb300091d) begin to address this challenge using transcription factor-based biosensors.

The authors demonstrate the application of these biosensors as a means to couple small-molecule production to either a detectable phenotype (fluorescent protein formation) or host fitness, termed here "synthetic selections". They then focus on a heterologous 1-butanol biosynthetic pathway, one with anticipated next-generation biofuel applications, to demonstrate the ability to couple microbial growth rate to product biosynthesis. The approach and results described here are broadly applicable in the field and can be readily extrapolated to nearly all microbially produced small molecules.

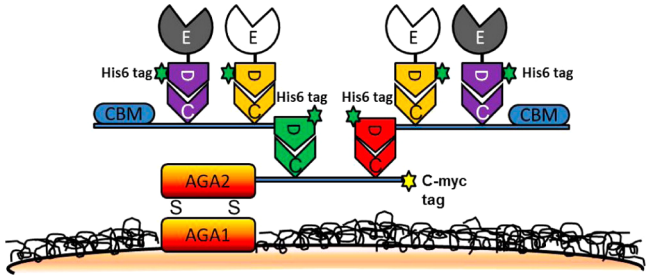
A BACTERIAL BIOSYNTHETIC PATHWAY TOOL



Synthetic biology attempts to produce natural products for use as pharmaceuticals or as chemical building blocks. Complex metabolic pathways needed to synthesize such compounds can be identified by computer-based methods. However, their cloning and functional expression in appropriate microbial hosts is usually very tedious or even impossible. Here, Loeschcke *et al.* (DOI: 10.1021/sb3000657) describe a newly developed system for the transfer and expression of biosynthetic pathways.

This system, named TREX, has the ability to transfer entire biosynthetic pathways, allowing for combinatorial metabolic engineering and thus for the identification and production of known and new metabolites in bacteria.

FUNCTIONAL DISPLAY OF COMPLEX CELLULOSOMES ON YEAST



A promising alternative to petroleum-based fuels, due to their sustainability and environment-friendly nature, are biofuels derived from lignocellulosic biomass. However, it is the lack of low-cost technology, needed especially for the hydrolysis of their highly ordered cellulose structure, that prevents the more widespread use of these fuels. Here, Tsai *et al.* (DOI: 10.1021/sb300047u) describe the development of a functional four-enzyme cellulosome on the yeast cell surface.

The authors demonstrated a 2-fold increase in ethanol production in cells displaying the tetravalent celluosome detailed here, as compared to those displaying a divalent cellulosome using similar enzyme amounts. This work highlights the importance of enzyme proximity on enhanced cellulose hydrolysis and, for the first time, describes the use of an adaptive strategy for the display of complex cellulosomes.

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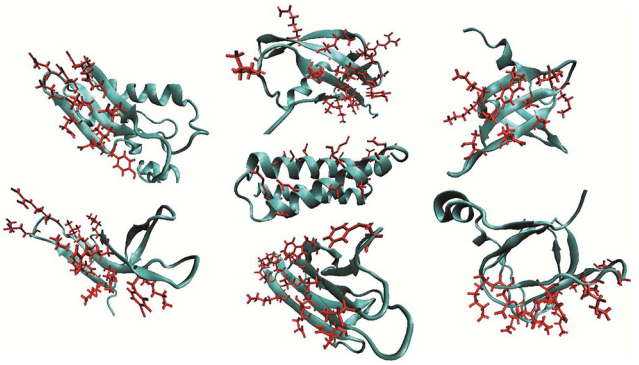
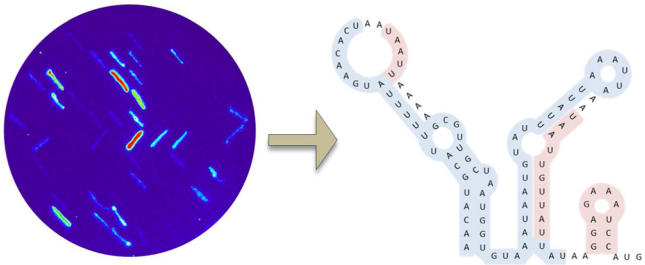
SCAFFOLD DIVERSIFICATION OF A HYPERTHERMOPHILIC PROTEIN SUPERLIBRARY



Antibodies are the most widely used binding proteins. More recently however, binding proteins have been generated by mutating specific regions on nonantibody proteins that act as scaffolds or "templates". Typically, a binding protein is isolated from a library of mutants derived from a single scaffold. Here, Hussain and Gera *et al.* (DOI: 10.1021/sb300029m) generated a "super-library" or "library-of-libraries" of ∼108 mutants by simultaneously mutating seven different scaffold proteins of hyperthermophilic origin.

Binders to a diverse set of target species, derived from multiple different scaffolds, were isolated from this library. Interestingly, the authors report that the affinities of binders obtained from the modestly sized superlibrary are comparable to those from a library with thousand-fold greater mutants, but derived from a single stable scaffold. Thus, scaffold diversification, or randomization of multiple different scaffolds, can be used as a powerful alternate strategy for combinatorial library construction.

NOVEL REGULATORY ELEMENTS IN *CHLAMYDOMONAS* CHLOROPLAST mRNA



Chloroplast gene expression is highly regulated during translation by sequence and secondary-structure elements in the 5′ untranslated region (UTR) of mRNAs. Specht and Mayfield (DOI: 10.1021/sb300069k) now present a synthetic biology approach using an arrayed oligonucleotide library to examine hundreds of designed variants of endogenous UTRs from *Chlamydomonas reinhardtii*, and quantitatively identify essential regions through next-generation sequencing of thousands of mutants.

The authors validate their strategy by characterizing the 5′ UTR of the psbD mRNA, and find that their analysis generally agrees with previous work identifying regions of importance. They, however, significantly expand and clarify the boundaries of these regulatory regions. The strategy is then used to characterize the previously unstudied psaA 5′ UTR to obtain a detailed map of regulatory regions. Finally, the authors create a novel synthetic UTR based on aggregate sequence analysis from the libraries and demonstrate that it significantly increases accumulation of an exogenous protein, attesting to the utility of this strategy for enhancing protein production in algal chloroplasts.

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