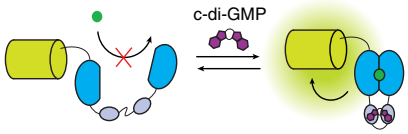
BIOSENSORS

Spotting the signal

ACS Chem. Biol. https://doi.org/10.1021/acschembio.7b01019 (2018)



Credit: ACS

Bacteria use 3′,5′-cyclic diguanosine monophosphate (c-di-GMP) as a signaling molecule to coordinate various processes including virulence and biofilm formation. Though a number of fluorescent biosensors exist to investigate c-di-GMP dynamics in cells, these tools are often not amenable to more complex environments. To address these issues, Dippel et al. developed chemiluminescent biosensors for c-di-GMP that avoid the need for external illumination. These protein-based biosensors consist of a PilZ domain inserted into a split luciferase fused to a fluorescent protein, such that binding of c-di-GMP to the PilZ domain enables luciferase activity and results in a fluorescence signal via bioluminescence resonance energy transfer. Because these biosensors are not hampered by autofluorescence, they can be used to assay c-di-GMP directly in lysates without further purification. Screening a library of diverse PilZ domains led to the identification of biosensor variants that are active and well-folded, exhibit high affinity and selectivity for c-di-GMP, and produce a large signal change upon binding. One of the resulting biosensors was used to develop an assay for diguanylate cyclase activity in *Escherichia coli*, and these biosensors could in the future be applied to the detection of c-di-GMP in complex biological systems or be adapted for the detection of other cyclic dinucleotides. *CD*

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MESSENGER RNA

Structure in sequence

Cell **173**, 181–195 (2018)

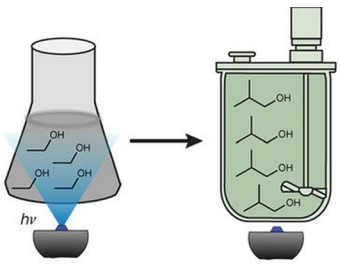
The folding of RNA molecules into specific secondary structures is essential for their function in various biological processes. Current understanding of endogenous mRNA structure and its variation across genes is limited, and is mainly based on theoretical speculation and in vitro experiments using synthetic genes. Mustoe et al. used the selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) strategy to obtain high-resolution structure models for approximately 200 endogenous mRNAs in *Escherichia coli*. They found that individual mRNAs adopt diverse, complex structures and contain a large number of conserved regulatory motifs. In addition, the authors observed that the mRNA structure is unstable in the cellular environment, largely because of translation-mediated structural destabilization, and that the structure of ribosome-binding sites plays a primary role in determining translational efficiency. Finally, they found that gene-linking mRNA structures mediate translational coupling of adjacent genes in a polycistron. Overall, this high-resolution structural atlas of endogenous mRNA provides deeper understanding of the diversity, dynamics and function of mRNA structure, reveals new connections between RNA structure and translation and enables predictions of potential new RNA-regulatory structural elements. *YS*

https://doi.org/10.1038/s41589-018-0050-3

METABOLIC ENGINEERING

Fueled by light

Nature **555**, 683–687 (2018)



Credit: Nature

Yeast normally metabolizes glucose into ethanol through a pyruvate decarboxylase (PDC)-dependent pathway. They can also produce the biofuel isobutanol by redirecting carbon flux through removal of PDC and overexpressing the isobutanol biosynthetic pathway, starting with acetolactate synthase (Ilv2). However, blocking ethanol production comes at the expense of reduced growth, suggesting that an approach that facilitates switching between ethanol and isobutanol synthesis might enable increased isobutanol yields. Zhao et al. utilized the light-sensitive transcription factor EL222 to switch yeast cells from synthesis of ethanol during illumination to production of isobutanol in the dark. They constructed an optogenetic circuit, OptoEXP, in which EL222 drives PDC1 expression in a PDC-deficient background, thus enabling cell growth and ethanol production in blue light. A second optogenetic circuit, OptoINVRT1, was constructed using EL222 to drive the GAL80 repressor, which inhibits ILV2 transcription under light conditions but induces it in the dark. Using periodic pulses of light to restore NAD^+ pools during isobutanol production, the authors produced 8.57 g/L of isobutanol in a laboratory-scale bioreactor. Overall, this optogenetic approach provides a method for improving metabolic engineering-derived yields using light. *GM*

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VACCINES

The peptide D-list

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The immune response to pathogens such as influenza virus involves the processing of pathogen proteins into peptides by antigen-presenting cells and the subsequent presentation of these antigenic peptides by MHC molecules to T cells. Administration of peptide-based vaccines, which are also presented via the MHC, can be difficult because of the instability of peptides. Miles et al. sought to generate an environmentally stable influenza vaccine by modifying an antigenic 9-mer peptide (GILGFVFTL) to contain all D-amino acids, but it was found to be nonimmunogenic. However, screening of a combinatorial peptide library for peptides that could mimic GILGFVFTL in eliciting a T-cell response identified an all-D peptide (gppqwnnpp) bearing no sequence resemblance to GILGFVFTL. This D-peptide had reduced potency due to decreased binding to MHC but was much more stable in human serum and under conditions that mimic gastric acid. This D-peptide had comparable MHC preference, could similarly amplify GILGFVFTL-specific human memory T cells in vitro, and could mobilize antigen-specific T-cell repertoires that closely mimic those elicited by GILGFVFTL. Finally, gppqwnnpp was immunogenic when injected into mice or when dosed orally and could protect the animals from an influenza challenge. These results highlight the benefits of D-amino acid engineering and provide a promising avenue toward more stable vaccines. MB

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