METABOLIC ENGINEERING

Strike that, reverse it

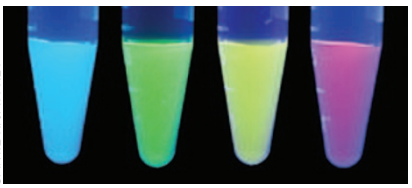
Nature **476**, 355–359 (2011)

Metabolic engineering of bacterial cells to generate commodity chemicals and biofuels typically entails either the rewiring of existing pathways to direct metabolic flux into desired endpoints or the identification of enzymes from across a host of bacterial species that can function at a given point within a proposed pathway. Dellomonaco *et al*. take a different approach: they envisioned that, by driving the known pathway for lipid degradation backwards, they could turn the normal products of this process—acetyl-CoA and propionyl-CoA—into substrates for a variety of short- and long-chain molecules. To accomplish this, the authors first introduced known mutations into two regulatory genes to cause constitutive expression of β-oxidation genes. It was also critical to inactivate two regulatory mechanisms that repress the same enzymes. Finally, the authors overexpressed several termination enzymes, specific for different molecules, to release the enzymatically bound or otherwise modified compounds. By feeding the cells glucose and in some cases propionate, the authors successfully obtained the expected products, including butanol and C16 and C18 fatty acids as well as several functionalized carboxylic acids. The high titers, yields and rates observed in the accumulation of these compounds were attributed to the direct usage of acetyl-CoA rather than to an activated version of the molecule. These results provide an intriguing example of the engineering of an endogenous pathway for a new use. *CG*

IMAGING

Green fluorescent RNA

Science **333**, 642–646 (2011)



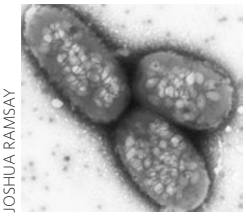
JEREMY S. PAIGE & SAMIE R. JAFFREY

Green fluorescent protein (GFP) has become a standard tool for visualization of proteins within cells and has inspired the engineering of a palette of fluorescent proteins that emit light at wavelengths across the visible spectrum. The fluorescent chromophores are assembled by cyclization of three amino acids at the core of the protein, and the properties of each chromophore are controlled by the protein environment immediately surrounding it. Paige *et al*. now expand the visualization toolbox to RNA through the identification of fluorescent RNA modules that comprise an RNA aptamer and a chromophore ligand. The authors synthesized a series of small-molecule analogs of the GFP chromophore and performed systematic evolution of ligands by exponential enrichment (SELEX) experiments to identify RNA sequences that selectively bound each of these compounds with high affinities. The chromophores were not fluorescent on their own or in cells but showed fluorescent properties when bound to a selected RNA aptamer. One dye-aptamer pair, which the authors called 'Spinach' because of its intense green color, displayed fluorescence properties similar to those of enhanced GFP and, unlike most fluorescent proteins, was resistant to photobleaching. To demonstrate the utility of these constructs, the authors genetically fused the RNA portion of Spinach to the 3′ UTR of a 5S noncoding RNA and used it to visualize the known trafficking pathway of this RNA in *Escherichia coli*. The identification and future optimization of these fluorescent RNA modules provides a new tool for visualizing RNA and its functions inside of cells. *TLS*

MICROBIOLOGY

Using gas as a raft

Proc. Natl. Acad. Sci. USA., published online 22 August 2011, doi:10.1073/pnas.1109169108



JOSHUA RAMSAY

The production of intracellular vesicles permeable to gases such as H_2, O_2, CO_2 or CH_4 has been shown to be important for buoyancy in various bacterial and archaeal species, but it has been mostly seen in planktonic microorganisms, whose physiology favors life at air-liquid interfaces. Ramsay *et al*. now show that the virulent Gram-negative bacteria Serratia 39906 also produces gas vesicles that serve a potentially important role in mobility to air-liquid interfaces. Using a transposon-based mutagenesis strategy, the authors found a gene cluster containing 19 open reading frames that is required to maintain an opaque colony phenotype, a typical measure of gas vesicle formation. Whereas a wild-type strain was buoyant and densely packed with gas vesicles, a strain lacking the gene cluster sedimented. The genes in the cluster were largely homologous to known gas vesicle proteins, such as a key structural protein GvpA, and to putative regulatory proteins. Monitoring the temporal expression patterns of *gvpA1* indicated that gas vesicle formation was stimulated under conditions of low O_2. Finally, the authors found that gas vesicle formation required the quorum sensing molecule *N*-butanoyl-L-homoserine lactone and RsmA, an RNA-binding protein that simultaneously represses flagellar motility. These results suggest that these bacteria use oxygen- and quorum-sensing–dependent gas vesicles to control mobility when an air-liquid interface lifestyle is desirable but flagellar-based motion is too energetically costly. *MB*

DEVELOPMENTAL BIOLOGY

Diffusing with Sog

Dev. Cell **21**, 375–383 (2011)

BMPs are secreted ligands that form concentration gradients across the dorsal-ventral (D/V) axis of developing tissues and thereby provide patterning information. Gradient formation is facilitated by modulators such as Chordin and Sog, homologous proteins that antagonize BMP signaling, or Tld, a metalloprotease responsible for signal degradation. Sog but not Chordin can also promote BMP diffusion; the biochemical foundation for this different biological activity is not understood. Now Peluso *et al*. demonstrate that BMP-dependent degradation of Sog but not Chordin could explain the difference. The authors identified three major Tld cleavage sites in Sog by sequencing degradation products; mutation of either of two sites retarded Sog degradation *in vitro* and mimicked Sog gain-of-function in *Drosophila* wing discs. Sog-i, a Sog variant with altered cleavage sites that can be cleaved independent of BMP, retained wild-type Sog-BMP interactions but decreased the net diffusive flux of BMP when expressed in wing discs. Sog-i expression broadened the spatial domain of active BMP signaling but resulted in a shallow-sloped concentration gradient, which resulted in cell fate changes along the D/V axis. Together these data indicate that residues in the Tld-recognition site render Sog cleavage dependent upon interaction with BMP, and that cosubstrate-dependent cleavage of Sog is important for BMP gradient formation and consequently D/V patterning. *AD*

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