ELECTROPHYSIOLOGY

Blinking bacteria

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AAAS

Bacterial processes ranging from metabolism to flagellar motion are driven by changes in membrane potential (V_m), yet little is known about the electrophysiology of individual bacterial cells because they are too small to be probed by conventional techniques. To monitor V_m in individual live *Escherichia coli* cells, Kralj *et al.* developed a genetically encoded indicator based on a proteorhodopsin called PROPS that they engineered to change fluorescence with fluctuations in V_m. After calibrating PROPS with known V_m values, they went on to discover that many cells showed quasi-periodic blinks in fluorescence independently of one another. The blink durations varied from cell to cell, ranging from 1 to 40 seconds, as did the intensities of the blinks. The blinks did not correlate with intracellular pH changes; instead, they were linked to electrical fluctuations (probably depolarization), and blinking corresponded to a loss of proton motive force. Additionally, the cells had to be alive and undergoing aerobic respiration to blink, and blinking was correlated with rapid efflux of a membrane-permeable dye. These results suggest that blinking might arise from a stress response and that efflux may be electrically regulated. *MB*

METABOLIC ENGINEERING

A plastic pathway

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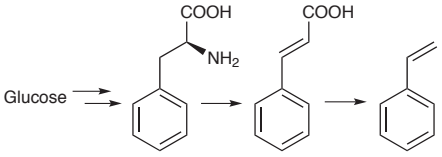

Styrene is an important precursor for a variety of polymer products. Its production from petroleum is highly energy intensive, so alternative synthetic routes are economically and environmentally desirable. Styrene can be produced naturally by yeast and plants, but specific biosynthetic routes have not been identified, and yields are too low to offer any commercial relevance. Metabolic engineering pathways producing related functionalized monoaromatic compounds have also been developed, but these have primarily used tyrosine as a precursor, resulting in phenolic products. Instead, McKenna and Nielsen envisioned converting phenylalanine to styrene by a two-step pathway of deamination and decarboxylation, with *trans*-cinnamic acid (tCA) as the intermediate. To identify a suitable deaminase, they screened a number of phenylalanine ammonia lyases (PALs) from different species and identified two *Arabidopsis thaliana* enzymes that could produce up to 918 mg L^{-1} tCA with no conversion of tyrosine. The authors then searched for a phenylacrylate decarboxylase, identifying one protein from *Saccharomyces cerevisiae*—FDC1—that could perform the reaction in high yields and with preference for tCA over the tyrosine-based intermediate. When both genes were introduced into an *E. coli* host, 260 mg L^{-1} styrene was detected in the aqueous phase, close to the observed toxicity limit of 300 mg L^{-1}. Beyond concerns of toxicity, the authors identified PAL turnover and availability of phenylalanine as possible limits to styrene production, pointing to several future directions to optimize output. *CG*

DNA DEMETHYLATION

TDG links in

Cell **146**, 67–79 (2011)

Thymine DNA glycosylase (TDG) is known to be a base excision repair protein involved in the removal of G•T mismatches, but recent studies have identified TDG as a regulator of transcription and DNA methylation. Cortellino *et al.* now clarify these roles by showing that TDG helps maintain a proper methylation state at CpG islands and is a component of a DNA demethylation-repair pathway that excises modified cytosine bases—5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC)—from genomic DNA. Using transgenic mouse technology and knockout-derived cell lines, the authors showed that *Tdg* null mutations produce embryonic lethality and developmental defects similar to retinoic acid signaling deficiencies. In both a transcriptional reporter system and developing embryos, TDG was shown to have an active role in demethylation of developmentally regulated genes, and an inactivating mutation at the glycosylase active site reproduced the embryonic lethality. Coimmunoprecipitation experiments revealed that TDG is associated with AID—a cytosine deaminase recently found to be involved in the conversion of 5hmC to 5-hydroxymethyluracil (5hmU) in genomic DNA—and GADD45, a stress response protein associated with demethylation. TDG's role in demethylation was dependent on its glycosylase activity, but in *vitro* assays showed that TDG removes T and 5hmU, but not 5mC or 5hmC, from DNA substrates. Taken together, these data argue that TDG functions as a component of a demethylation complex that removes modified cytosine bases by a sequential deamination and base excision repair pathway. *TLS*

PROTEIN-PROTEIN INTERACTIONS

GRBing dynamic networks

Nat. Biotechnol. **29**, 653–658 (2011)

Adaptor proteins such as GRB2 are hubs for protein-protein interactions (PPIs) that convey signals from cell surface receptors to downstream effectors. Understanding the dynamics of these signaling networks in stimulated cells remains challenging. Bisson *et al.* now report affinity purification–selected reaction monitoring (AP-SRM) as a new MS–based approach to evaluate context- and time-specific PPIs with GRB2 after growth factor stimulation. To validate the method, the authors show that the function of SH2 or SH3 is necessary to detect proteins known to interact with GRB2 via these domains. The authors applied AP-SRM to quantify the interaction between GRB2 and 90 signaling proteins upon activation with epidermal growth factor in a time-dependent manner, identifying three groups of proteins that bind GRB2 with distinct kinetics. Finally, the authors quantified PPIs upon activation with one of six different growth factors to identify the core GRB2 machinery, which includes proteins that interact with GRB2 under multiple conditions as well as growth factor–specific PPI networks. Compared to other MS strategies, AP-SRM is highly sensitive, reproducible and quantitative, and it does not require *in vivo* labeling. Thus, AP-SRM provides a robust alternative for hypothesis-driven investigations to quantitatively measure the dynamics of PPI networks. *AD*