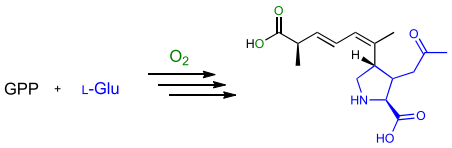
BIOSYNTHESIS

Devilish diatoms

Science **361**, 1356–1358 (2018)



The neurotoxin domoic acid (DA) is produced by marine diatoms of the genus *Pseudo-nitzschia*, which can cause harmful algal blooms. Past work suggested glutamic acid and geranyl pyrophosphate as precursors of DA, but the proteins and transformations involved in its biosynthesis were not known. Using transcriptome sequencing to identify genes that were upregulated during growth with limited phosphate and increased carbon dioxide levels (conditions known to promote DA production), Brunson and McKinnie et al. have now elucidated the biosynthetic pathway of this environmental neurotoxin. Among the upregulated genes was a four-gene cluster encoding a predicted terpene cyclase (DabA), α-ketoglutarate-dependent dioxygenase (DabC), cytochrome P450 (DabD), and a hypothetical protein (DabB). In vitro reconstitution of these enzymes validated that DabA catalyzes N-geranylation of L-glutamate to form *N*-geranyl-L-glutamic acid (L-NGG), which is successively oxidized by DabD into 7′-carboxy-L-NGG and cyclized by DabC into isodomoic acid A. Although an enzyme responsible for the final isomerization step to DA was not identified in this study, the discovery of the other biosynthetic genes may help to better understand the conditions that promote algal blooms and monitor DA production capability during those events. *CD*

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BACTERIAL PATHOGENESIS

Metal Enterococcus equipment

PLoS Pathog. **14**, e1007102 (2018)

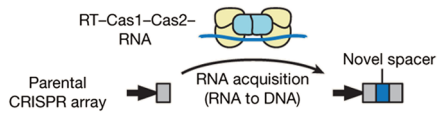
Pathogens such as *Enterococcus faecalis* require manganese (Mn) for growth and virulence, but the host can restrict the Mn levels available to the bacteria using metal-binding proteins such as calprotectin. To determine how *E. faecalis* is able to acquire Mn for growth, Colomer-Winter et al. performed genomic profiling using known streptococci Mn transporter sequences and identified three potential *E. faecalis* Mn transporters: EfaCBA (an ABC-type transporter), MntH1 and MntH2 (Nramp-type transporters). Testing the virulence of a panel of single-, double- and triple-deletion mutant strains, their ability to grow in Mn-restricted media and their susceptibility to inhibition by calprotectin, the authors determined that EfaCBA and MntH2 are the primary Mn transporters required for virulence in two different mammalian systems. Quantification of cellular Mn content of the mutants showed that all three transporters could effectively transport Mn and that the growth defect of the triple mutant is primarily due to its inability to acquire Mn. EfaCBA and MntH2 could also each support *E. faecalis* growth and biofilm formation in Mn-limiting conditions such as human serum or urine. These results reveal a new strategy by which *E. faecalis* is able to overcome the nutritional immunity imposed by mammalian hosts. *MB*

https://doi.org/10.1038/s41589-018-0173-6

GENETIC ENGINEERING

Transcriptional recorder

Nature **562**, 380–385 (2018)



Credit: *Nature*

Microbial CRISPR–Cas systems can integrate short foreign DNA sequences into a host's genome, recording a memory of infection for use in future immune responses. These acquired sequences, called spacers, are separated by direct repeats and constitute CRISPR arrays. Though acquisition of CRISPR spacers from RNA has been found in some marine organisms, it is not clear whether this mechanism can be expanded to other organisms. Schmidt et al. found that Cas1–Cas2 from *Fusicatenibacter saccharivorans* (*Fs*RT–Cas1–Cas2) was able to capture and convert RNAs into DNA spacers in *Escherichia coli*. By sequencing the CRISPR arrays, they found that RNA-derived spacers captured by *Fs*RT–Cas1–Cas2 were mainly from AT-rich regions at the ends of transcripts and were positively correlated to the cumulative transcript abundance. This method, termed Record-seq, enabled quantitative recording of transcriptome-wide alterations under oxidative and acid-stress-stimulated conditions. Notably, Record-seq succeeded in recording the historical transcriptional changes induced by transient paraquat exposure, whereas traditional RNA-seq failed due to the recovered transcriptional level after paraquat removal. This study provides a useful tool for molecular recording and paves a way for transcriptional lineage tracking. *YS*

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DNA EPIGENOMICS

Failure to convert

Nat. Biotechnol. https://doi.org/10.1038/nbt.4204 (2018)

5-Methylcytosine (5mC) undergoes DNA demethylation through TET-mediated oxidation, in the process forming the stable intermediate 5-hydroxymethylcytosine (5hmC), which is enriched in neurons and embryonic stem cells. Detection of 5hmC at single-base resolution relies on adapting sequencing methods that utilize bisulfite to chemically convert cytosine and 5mC to thymidine but leave 5hmC protected from conversion. However, bisulfite also induces significant degradation of genomic DNA, preventing analysis of rare cell types. To address this limitation, Schutsky et al. developed an enzymatic method called APOBEC-coupled epigenetic sequencing (ACE-seq). Building on prior biochemical evidence that AID/APOBEC DNA deaminases can discriminate between modified cytosine bases, they utilized APOBEC3A to enzymatically convert cytosine and 5mC, but not 5hmC bases. Testing of ACE-seq on phage genomes showed accurate detection of >98.5% of 5hmC bases, with cytosine and 5mC nonconversion rates of <0.5%, outperforming existing bisulfite-dependent methods without impacting DNA stability. ACE-seq analysis of purified cortical excitatory neurons revealed that specific genomic features can differ greatly in 5hmC content, with imprinted regions depleted for 5hmC and enhancers highly enriched. Overall, ACE-seq provides a reliable new method to detect 5-hmC with high confidence. *GM*

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