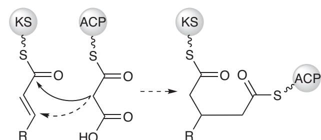
BIOSYNTHESIS

Branch point

Nature, doi:10.1038/nature12588



Polyketide synthase machinery has, to this point, exclusively used head-to-tail connectivity to attach incoming monomers to the ends of growing polyketide chains (solid arrow). A new study from Bretschneider *et al.* overturns this dogma, reporting that an acyl carrier protein (ACP)-bound monomer can attack the vinylogous position of an α,β-unsaturated chain, introducing a branch point (dashed arrows). The authors' previous study of the rhizoxin biosynthetic pathway indicated that the connectivity of the molecule could not be rationalized by the known protein modules in the assembly line. To elucidate the pathway, the authors focused on an unusual ketosynthase-branching didomain found in the RhiE enzyme. *In vitro* reconstitution of the didomain, its ACP partner and other requisite proteins, along with chemical synthesis of activated substrates, provided direct evidence that a Michael addition was indeed occurring. NMR characterization of enzyme-bound, labeled substrates confirmed that C-C bond formation precedes release of the chain from the ketosynthase (KS) domain via an intramolecular esterification reaction; by synthetically removing the hydroxyl group necessary for the esterification, the authors were able to observe the doubly-bound intermediate. Surprisingly, structural and mutational evidence suggested the ketosynthase performs all reaction steps, while the new branching domain does not play a catalytic role, leaving the mechanism of this intriguing reaction to be resolved. *CG*

METABOLISM

A clockwork burn

Science, doi:10.1126/science.1243417

The circadian clock regulates diverse facets of our behavior and physiology through tight transcriptional control of activators and repressors. Mice deficient in core clock components such as Clock have recently defined links to metabolic regulation, though the molecular machinery that ties the circadian clock to metabolic homeostasis is unclear. Peek *et al.* now report that mouse mutants in the activator loop of the clock (Clock and Bmal1) cause a decrease in fatty acid oxidation, oxygen consumption and NAD^+ levels, indicating that mitochondrial oxidative reactions are under strict circadian control. As NAD^+ levels are known to undergo cycling owing to circadian regulation of the biosynthesis enzyme, NAMPT, the authors hypothesized that the defective biosynthesis of NAD^+ might be the primary cause for the loss of metabolic regulation. To test this hypothesis, they restored cellular NAD^+ levels by providing nicotinamide mononucleotide, which elevated fatty acid oxidation and oxygen consumption to functional levels in intact animals and isolated mitochondria. NAD^+ can promote fatty acid oxidation by regulating the activity of SIRT3, a mitochondrial deacetylase. Consistent with this, SIRT3 activity was diminished in Bmal1-deficient mice, resulting in increased acetylation of mitochondrial oxidative enzymes. Replenishing NAD^+ levels in Bmal1-deficient mice could restore SIRT3 activity, resulting in the deacetylation and increased activity of fatty acid oxidation enzymes. These findings suggest that the circadian regulation of NAD^+ levels is a crucial rheostat to control mitochondrial function and maintain metabolic homeostasis. *GM*

OPTOGENETICS

Shine a LITE

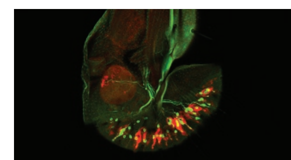
Nature, doi:10.1038/nature12466

The ability to spatially and temporally regulate endogenous gene expression is essential to elucidate gene function. Konermann *et al.* developed a system called light-inducible transcriptional effectors (LITE), which can either activate or repress gene expression through the application of light. LITE uses two distinct components: a TALE DNA-binding domain recognizing specific promoter elements bound to the light-sensitive cryptochrome 2 (CRY2) protein and the CRY2 partner protein CIB1 fused to a transcriptional effector—in this case, the activator VP64. Application of blue light stimulates the dimerization of CRY2 and CIB1, bringing VP64 to the promoter region, which results in the initiation of transcription. LITE-mediated gene induction was rapid, with increased gene expression detected within 30 min after light stimulation and with a half-life of 3 h. Next, the authors developed an adeno-associated virus vector to introduce LITE constructs into primary cortical neurons activating an array of endogenous neuronal genes through the delivery of light. Interestingly, the authors could also modulate *in vivo* gene expression specifically in the prefrontal cortex by introducing light through a fibre optic cannula. In addition to controlling gene expression, the authors were able to elicit light-mediated epigenetic changes by using the histone-modifying domains, such as mSin3 (SID4X), to inhibit gene expression through reduction of H3K9 acetylation. Taken together, LITE may be an efficient approach to precisely control mammalian gene expression in specific cell types. *GM*

NEUROSCIENCE

Bitter oblivion

Neuron **79**, 725–737 (2013)


CRAIG MONTELL

The chemical composition of food dictates gustatory responses (attraction and preferences for food) among animals by regulating taste cells expressing cell surface gustatory receptors (GRs). Aversive responses to quinine and other bitter chemicals are seen in many organisms, and there are two mechanisms through which bitter compounds inhibit the sweet taste within the gustatory system. One involves competition between different gustatory receptor neurons (GRNs) that are activated by bitter compounds and sugars and either inhibit or stimulate feeding, respectively. The gustatory sensilla (bristles) that house the GRNs also contain accessory cells (thecogen cells) that express odorant-binding proteins (OBPs). Jeong *et al.* focused on four OBPs that they had found to be enriched in gustatory sensilla of the main taste organ. Mutating one, OBP49a, caused flies to be oblivious to bitter compounds that had been mixed with their sucrose meal, but even mutant flies behaved normally to either compound type alone. Measuring nerve firings elicited by tastants in mutant flies indicated that OBP49a is required for direct suppression of GRNs that are activated by sugars. A fluorescent protein complementation assay showed a close association between OBP49a and one GR required for sucrose detection, GR64a.These results and additional tests of cell-autonomous function as well as a demonstration by SPR that bitter chemicals bind OBP49a suggest that OBP49a acts directly at the cell surface of sugar-responsive GRNs to inhibit action potentials in response to bitter compounds. *MB*

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