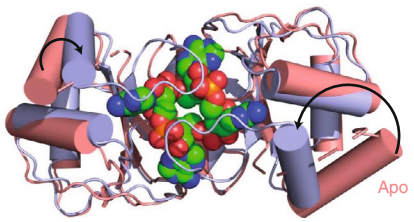
CRISPR-CAS

A stop to signaling

Nature **577**, 572–575 (2020)



Credit: *Nature*

Bacteria use CRISPR–Cas as an adaptive immune system against viral infection and exogenous nucleic acids. In particular, type III CRISPR–Cas systems can synthesize cyclic oligoadenylates as secondary messengers which bind and activate nucleases to degrade viral nucleic acids. Accordingly, viruses have evolved anti-CRISPR systems (Acr) as a response. Athukoralage et al. identified the DUF1874 protein family as an Acr ring nuclease that degrades cyclic tetraadenylate (cA4). A virus lacking the *duf1874* gene lost its infectious ability in an engineered bacterial strain selectively expressing the type III CRISPR–Cas system, but viral infection could be recovered by deletion of the cA4-activated effector Csx1. Structural characterization of the DUF1874 protein in complex with cA4 revealed that cA4 binds in a central pocket, inducing the movement of a loop and helix that buries cA4 inside. The essential catalytic residue His47 of each monomer is positioned toward the 2′-hydroxyl group in cA4 for nucleophilic attack. These findings add a new family to known Acr systems and reflect the continuing evolution of viral evasion strategies. *YS*

https://doi.org/10.1038/s41589-020-0488-y

LIPIDS

Investigating PIs

ACS Chem. Biol. **15**, 33–38 (2020)

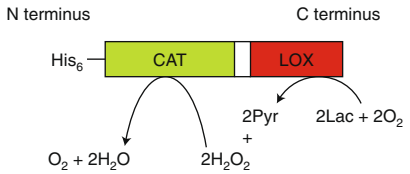
Phosphoinositides (PIs) such as PI(4,5)P_2 are important phospholipids that act as anchoring molecules for signaling proteins at the plasma membrane (PM) and as second messengers downstream of growth factor receptors, as is the case for PI(3,4,5)P_3. To expand the PI biosensor toolbox, Hertel et al. generated PlcR, whose sensing unit, which is flanked by FRET donor and acceptor fluorophores, is based on a PI(4,5)P_2-binding PH domain and a negatively charged pseudoligand that can bind to the PI-binding pocket of this domain. Displacement of the pseudoligand by PI(4,5)P_2 induces a conformational change that the authors could monitor by real-time FRET changes. Using the same sensing unit, they next generated dPlcR, which has at its ends a dimerization-dependent green fluorescent protein pair that increases fluorescence in the presence of PI(4,5)P_2. Similarly, the authors generated dInPAkt with a dimerization-dependent red fluorescent protein pair to monitor 3-phosphoinositides, including PI(3,4)P_2 and PI(3,4,5)P_3, generated by PI3K downstream of growth factor receptors. The combination of dlnPAkt with dPlcR led to the conclusion that PI(3,4,5)P_3 production is correlated with PI(4,5)P_2 depletion and that a substantial fraction of PI(4,5)P_2 is unavailable to PI3K. These tools should prove useful for uncovering real-time PI signaling dynamics in living cells. *MB*

https://doi.org/10.1038/s41589-020-0487-z

REDOX BIOLOGY

Restoring the balance

Nat. Biotechnol. https://doi.org/10.1038/s41587-019-0377-7 (2020)



Credit: *Nat. Biotechnol.*

Reductive stress in mitochondrial disease results from an abundance of reducing equivalents in the cell, such as an elevated NADH:NAD^+ ratio, which impairs NAD^+-dependent pathways and generates reactive oxygen species. Decreasing the extracellular lactate:pyruvate ratio can lower the intracellular NADH:NAD^+ ratio, but directly adding stoichiometric amounts of pyruvate to achieve this is not a feasible clinical treatment for mitochondrial disorders. To target the lactate:pyruvate ratio enzymatically, Patgiri et al. constructed LOXCAT, a fusion of lactate oxidase, which converts lactate and O_2 to pyruvate and H_2O_2, with catalase to detoxify the H_2O_2 byproduct by converting it to water and O_2. In vitro, LOXCAT indeed converted lactate to pyruvate without detectable H_2O_2 leakage. In cells, under antimycin-induced reductive stress, media-supplemented LOXCAT lowered both the extracellular lactate:pyruvate ratio and the intracellular NADH:NAD^+ ratio and rescued impaired glycolysis and proliferative defects. When injected into blood, LOXCAT was able to restore heart and brain NADH:NAD^+ balance in a mouse model of mitochondrial drug toxicity. By demonstrating the successful catalytic modulation of lactate:pyruvate ratios, LOXCAT provides a tool for the study and potential treatment of redox dysregulation. *CD*

https://doi.org/10.1038/s41589-020-0485-1

CANCER METABOLISM

Hooked on vitamins

Cancer Cell **37**, 71–84 (2020)

Different cancer cell types utilize specific metabolic pathways to promote their growth and proliferation. To identify metabolic dependencies required for the growth of acute myeloid leukemia (AML), Chen et al. performed a CRISPR–Cas9 screen revealing that pyridoxal kinase (PDXK), an enzyme that mediates the production of pyridoxal phosphate (PLP) from vitamin B6, was selectively depleted in leukemia cancer cells and was specifically required for AML cell proliferation. Metabolomic profiling of PDXK-depleted AML cells showed decreased nucleotide and glycolytic metabolites, confirming PLP as an essential co-factor for enzymes involved in nucleic acid and lipid metabolism. Given that the loss of PDXK activity reduced PLP levels in all cell types but only impacted AML proliferation, the authors performed a focused CRISPR–Cas9 screen and identified eight PLP-dependent enzymes required for AML proliferation. In particular, they found that ODC1, a decarboxylase that promotes putrescine production, and GOT2, a transaminase that generates aspartate and nucleotides, were essential for regulating leukemic proliferation. Finally, treatment of a mouse leukemic model with a PDXK inhibitor reduced leukemic cell growth. Overall, these findings will inspire improved drug discovery efforts targeting PDXK to treat AML. *GM*

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