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RAF Inhibitors, Fishing Bacterial Transporters Out of Metagenomes, and Yeast-Based, Industrial Scale Production of Isoprenoids

Every month the editors of *Cell Chemical Biology* bring you highlights of the most recent chemical biology literature. Our October 2016 selection includes systematic structural, biochemical, and cellular characterization of B-RAF inhibitors; connecting bacterial transporters with their physiologically relevant ligands; and rewiring yeast metabolism for industrial scale production of isoprenoids.

Smoothing the Issues around RAF

RAF kinases are a family of serine/threonine protein kinases that includes three RAF isoforms: A-RAF, B-RAF and C-RAF. RAF kinases are components of RAS-RAF-MEK-ERK signaling pathway, where RAF kinases participate as dimers to activate ERK signaling. Mutations in RAF kinase genes are associated with different types of cancer; for example, B-RAFV600E is a mutation found in more than 50% of melanomas. Inhibitors of B-RAFV600E have been in clinical use and different stages of preclinical and clinical development. Although the effects of RAF inhibitors are well documented, a mechanistic basis for the observed activity has not been previously reported.

Karoulia et al. examine the effects of eight B-RAF inhibitors and show that distinct allosteric regulation mechanisms are at work. The key structural feature that determines the response of B-RAF to inhibitor binding is the position of the αC helix. Inhibitors that stabilize the αC helix in IN position are compatible with B-RAF dimer formation, promote maximal RAF binding to RAS (RAF priming), and are able to effectively inhibit B-RAF dimer activity. On the other hand, inhibitors that result in the αC helix movement towards the OUT position can occupy only one protomer within the B-RAF dimer, promote minimal RAF priming, and in effect act as negative allosteric regulators of inhibitor binding in the second protomer, leading to resistance to inhibition. This suggests that αC-IN inhibitors may effectively inhibit RAF dimers, whereas αC-OUT inhibitors are only effectively inhibit RAF monomers. Overall, the key insight that Karoulia et al. deliver is that developing next-generation RAF inhibitors will require paying close attention to the structural effects induced by the inhibitor and RAF dimerization regulated by the cellular context. Therefore, both OUT and IN inhibitors may have a role to play in the final RAF targeting arsenal.

Karoulia et al. (2016). Cancer Cell 30, 485–498. http://dx.doi.org/10.1016/j.ccell.2016.06.024

Yeasty Treats on Industrial Scale

Saccharomyces cerevisiae, also known as brewer's yeast or baking yeast, has been a powerful industrial microorganism for centuries in processes such as beer brewing, wine making, and baking. Biotechnologically speaking, the core application of *S. cerevisiae* comes in the form of ethanol production, and over more recent history, there has been a growing interest in using engineered *S. cerevisiae* for production of other commodity chemicals. One of the prominent examples is transgenic yeast engineered to produce artemisinic acid, an isoprenoid precursor of a high-value antimalarial compound, artemisinin.

***Saccharomyces cerevisiae* is one of the most useful microbes at our disposal. It not only serves as a powerful model organism for basic biological research, but it is used in many everyday applications, such as baking, as well as on an industrial scale in brewing. The cutting edge of yeast biotechnology is currently trying to determine how to use this versatile microbe to produce high-value chemical commodities, as illustrated by the β-farnesene example presented by Meadows et al.**

Meadows et al. build on that success to further improve the yield of industrial-scale production of a sesquiterpene, β-farnesene. The main challenge for Meadows et al. was to figure out how to use the existing central metabolism of yeast yet modify it in a way that enables most efficient possible utilization of both oxygen and carbon feedstock, two primary expenses in industrial fermentation. This approach requires careful metabolic pathway modeling and engineering. In the end, Meadows et al. achieve improvements by adding four enzymes—xylulose-5-phosphate specific phosphoketolase (xPK), acetaldehyde dehydrogenase (ADA), NADH-consuming HMG-CoA reductase (NADH-HMGr), and phosphotransacetylase (PTA)—into the yeast, bringing them very close to desired performance. In this case, Meadows et al. borrow ADA from *Dickeya zeae*, PTA from *Clostridium kluyveri*, xPK from *Leuconostoc mesenteroides*, and NADH-HMGr from *Silicibacter pomeryoi*, highlighting the importance of enzyme biodiversity to those who work on metabolic engineering and development of strains for industrial-scale production. The strains developed by

Meadows et al. are able to function in 200,000 L industrial bioreactors and reliably produce β-farnesene at a scale that, to those of us who experience science at much smaller scales, borders on science fiction.
Meadows et al. (2016). Nature 537, 694–697. http://dx.doi.org/10.1038/nature19769

From Metagenomes to New Bacterial Membrane Transporters

Although they play major physiological roles, many bacterial membrane transporters are yet to be functionally annotated and linked to the specific substrates that they transport. One large obstacle to a better understanding of bacterial membrane transporters is the challenge of culturing many of the environmental bacteria, which leaves us in the dark regarding vast spaces of the metagenome.

This is what makes the approach taken by Genee et al. so exciting. In their recent paper, the authors describe a riboswitch-based biosensor system that allows them to screen large libraries of metagenomic DNA from soil and gut bacteria to identify unknown transporters involved in the transport of a small molecule ligand that responds to the riboswitch. In the example Genee et al. use to validate their approach and illustrate its utility, the authors focus on identifying thiamine (vitamin B1) transporters using ThiM19, a thiamine pyrophosphate (TPP)-responsive riboswitch, in combination with engineered *E. coli* strains in which growth under dual selection criteria is only possible upon TPP binding to ThiM19. They transform the engineered *E. coli* strain with metagenomic DNA libraries and look for the strains that are able to grow and form colonies on plates in the presence of 0.5 μM of thiamine. A closer look at the genome segments led to the discovery of nicotinamide riboside and nicotinamide mononucleotide transporter genes (PnuC) as the likely thiamin-uptake transporters, which was confirmed by follow-up functional studies and resulted in annotation of a new class of bacterial thiamine transporters, PnuT. Genee et al. include another example in which the biosensor contains a synthetic aptamer generated via SELEX procedure, confirming that their strategy is equally successful when employing a fully synthetic riboswitch.
Genee et al. (2016) Nature Chem. Biol. Published October 3, 2016. http://dx.doi.org/10.1038/nchembio.2189

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