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Spotlight

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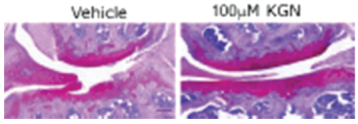
CREATING CARTILAGE

Osteoarthritis, a disease characterized by the breakdown of cartilage in the joints, is estimated to affect approximately 10% of the world's population over 60 years of age, and few therapeutic options are available to treat this painful condition. Mesenchymal stem cells (MSCs) are a special type of precursor cell that can mature into chondrocytes, the cells that make cartilage. MSCs reside in cartilage and therefore present an intriguing opportunity to repair the damaged tissue in osteoarthritis. What is needed are methods to direct MSCs to become chondrocytes. Now, Johnson *et al.* (*Science*, published online April 5, 2012; DOI: 10.1126/science1215157) report the discovery of a small molecule called kartogenin that promotes the differentiation of MSCs into chondrocytes.

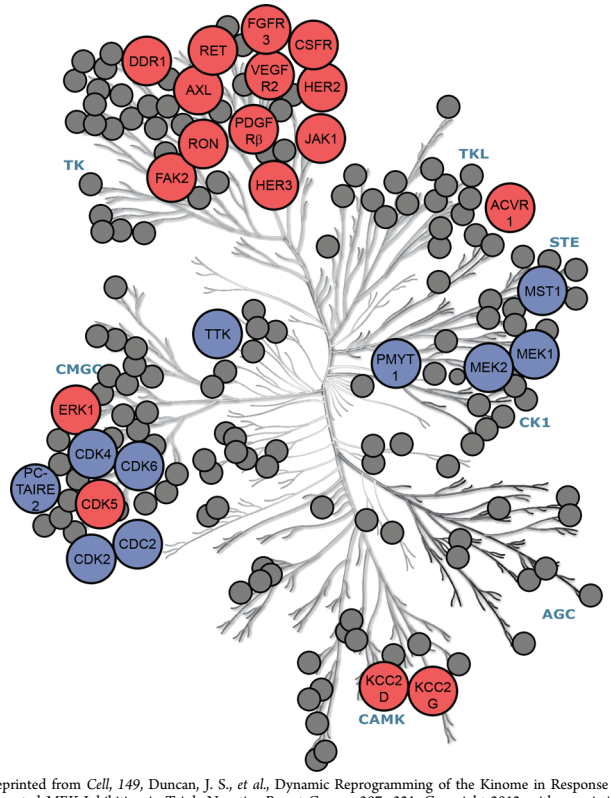


From Johnson, K., *et al.*, *Science*, April 5, 2012; DOI: 10.1126/science1215157. Reprinted with permission from AAAS.

Kartogenin was identified from over 20,000 small molecules screened for their ability to promote chondrocyte differentiation from MSCs in a cell-based, high throughput assay. Testing in *in vitro* and animal models demonstrated that kartogenin also protected cells from cytokine-induced damage that occurs during osteoarthritis; that injection of kartogenin directly into the diseased joint of a mouse resulted in the regeneration of cartilage; and that treatment of mice with kartogenin resulted in a reduction in several indicators of osteoarthritis, including joint score, circulating type II collagen fragments, and pain. To determine the mechanism of action of kartogenin, its molecular target was identified using a biotinylated kartogenin derivative containing a photocross-linking group. The protein filamin A, an actin-binding protein, was identified as kartogenin's target. Further probing revealed that kartogenin blocks the interaction between filamin A and a transcription factor called core-binding factor β (CBFβ) subunit. This results in the translocation of CBFβ to the cell nucleus, where it activates a transcription factor called RUNX1 that ultimately directs chondrocyte differentiation. Kartogenin is an exciting jumping off point for the further discovery of drugs that could promote cartilage generation and is also a valuable discovery tool for probing chondrocyte biology. **Eva J. Gordon, Ph.D.**

RATIONAL COMBINATION THERAPY DESIGN

Many of the molecular pathways that orchestrate cancer development involve kinases, making them attractive anticancer targets. However, the effectiveness of a single kinase inhibitor as a cancer therapy is often disrupted by the tumor's ability to develop resistance to the drug. In many tumor types including triple negative breast cancer, a type of breast cancer that is notoriously difficult to target and often aggressive, evidence suggests that resistance develops from the activation of other kinase pathways in the cancer cells that are able to circumvent the drug's actions. Such resistance can be limited by treatment with multiple kinase inhibitors, but determining which combination of kinases to target is a formidable challenge. Now, Duncan *et al.* (*Cell* 2012, *149*, 307−321) report the use of a quantitative chemical proteomics approach to assess changes in kinome activity in cell lines and mouse models of triple negative breast cancer in response to inhibition of the kinase MEK.



Reprinted from *Cell*, *149*, Duncan, J. S., *et al.*, Dynamic Reprogramming of the Kinome in Response to Targeted MEK Inhibition in Triple-Negative Breast Cancer, 307−321. Copyright 2012, with permission from Elsevier.

This comprehensive analysis of changes in kinome dynamics in response to MEK inhibition was achieved by integrating information gained from three main techniques: RNA-seq, which allowed determination of the kinome transcript expression profile; affinity capture and mass spectrometry analysis, which enabled profiling of kinase activity; and RNAi, which enabled evaluation of the roles of the kinases that were activated in response to the MEK inhibitors. Together, these analyses of kinome dynamics facilitated generation of a kinome response signature and prediction of a potential kinase inhibitor combination that might more effectively target triple negative breast cancer. Indeed, the combination therapy, which included the MEK inhibitor AZD6244 and the receptor tyrosine kinase inhibitor sorafenib, promoted tumor apoptosis and regression in mouse models of triple negative breast cancer not seen with either drug alone. In addition to providing insight into potential combination therapies for triple negative breast cancer, this innovative systems kinome approach can be extended to the

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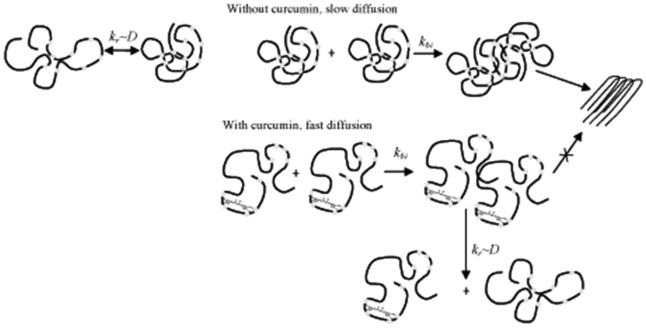
rational design of combination therapy for various other tumor types. **Eva J. Gordon, Ph.D.**

RECONFIGURING PROTEIN AGGREGATION

Deposits of aberrantly aggregated proteins are hallmarks of several neurodegenerative disorders including Alzheimer and Parkinson diseases. Investigation of the mechanisms involved in the aggregation process will help in the development of new strategies to prevent it. Studies aimed at finding inhibitors of α-synuclein aggregation, which is implicated in Parkinson disease, have focused on blocking the aggregation of oligomeric species, in part because they are easier to monitor than their monomeric counterparts. Now, Ahmad and Lapidus (*J. Biol. Chem.* 2012, *287*, 9193−9199) investigate the mechanism by which the small molecule curcumin, a compound with known medicinal properties that is found in the Indian spice turmeric, prevents α-synuclein aggregation through its interaction with the monomeric species.

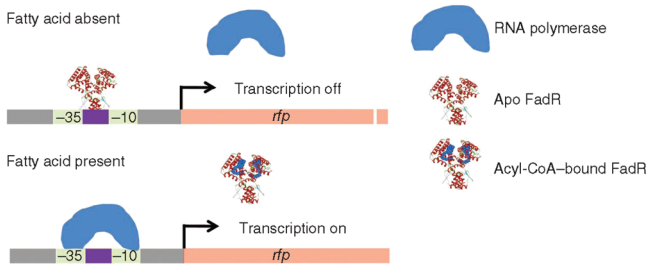


Ahmad, B. and Lapidus, L. J., *J. Biol. Chem*, 2012, *287*, 9193−9199. Copyright 2012 The American Society for Biochemistry and Molecular Biology.

Recent evidence suggests that α-synuclein normally exists as a helical tetramer in human cells that is not particularly prone to aggregation. It is thought that the first step in the aggregation of α-synuclein is dissociation of the tetramer to disordered monomers, which then can aggregate. This process is controlled by the rate at which one part of the protein randomly interacts with another part, called the reconfiguration or intramolecular diffusion rate. Using a variety of optical absorption and fluorescence methods, it was found that curcumin binds to monomeric α-synuclein and completely prevents its aggregation. Moreover, the compound appears to affect the global dynamics of α-synuclein by increasing the reconfiguration rate of the protein. This allows exposed hydrophobic portions of the protein, which could aggregate with neighboring proteins if given enough time, to reconfigure into a more stable intramolecular conformation instead. These results offer insight into protein aggregation dynamics and suggest an exciting new strategy for development of protein aggregation inhibitors. **Eva J. Gordon, Ph.D.**

PROGRAMMING MICROBES TO CHURN OUT BIODIESEL

As researchers look for ways to produce chemicals with less energy and waste, microbes present one attractive option. But scientists still needed to answer a tricky question: How do you prime the metabolism of these organisms to churn out compounds in high yields? Jay Keasling and his colleagues (Zhang *et al.*, *Nat. Biotechnol.* 2012, doi:10.1038/nbt.2149) present a new solution to that problem: a dynamic sensor-regulatory system for producing biodiesel compounds that both senses intermediates in the cell and regulates gene expression to optimize product yields.



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To develop this system for producing fatty acid ethyl esters (FAEEs), the researchers first needed to develop a sensor system for key compounds in the pathway, fatty acid-CoA and free fatty acids. They used a naturally occurring fatty-acid sensing protein and transcription factor, FadR, and inserted its 17-bp DNA binding sequence so that it overlapped with the RNA polymerase binding domain upstream of a gene that encoded red fluorescent protein. In the absence of fatty acids, transcription was blocked. They then used added promoters to optimize the response of the sensor. In a strain of fatty acid producing cells transfected with the plasmid, cells glowed red in response to the production of fatty acids.

With the sensor in place, the researchers built a set of modules that would allow the sensor to regulate fatty acid production. Previously the Keasling group had reported a biosynthetic pathway for generating these compounds that contains 3 modules: one for producing fatty acids, a second for producing ethanol, and a third that converts these products to an ester. Because ethanol is toxic to the cell and acyl-CoA formation requires significant cellular resources and is reversible, the researchers placed these last two components under the regulation of the sensor.

Cells using this system produced three times as much FAEE as previously reported, nearly 30% of the maximum theoretical yield. By pairing a natural or engineered biosensor with almost any biosynthetic pathway, the researchers have developed a general strategy for maximizing microbial production of customized chemicals. **Sarah A. Webb, Ph.D.**

A QUERY OF THE QUORUM

Even tiny bacteria have a way of talking to their neighbors. Chemical signals produced by many prokaryotes play a role in the cell-to-cell communication known as quorum sensing. Among the best characterized signals between bacterial communities and even proposed to mediate interspecies crosstalk are the Autoinducer-2 compounds. These molecules are derived from 4,5-dihydroxy-2,3-pentanedione (DPD), a hydrated five-carbon chain that was previously shown to exist in constant equilibrium between a linear form and either of two cyclic isomers. Previous studies demonstrated that under acidic conditions, the equilibrium was shifted toward the cyclic isoforms with a 4:1 ratio to the linear form. Now, Globisch *et al.* (*Angew. Chem., Int. Ed.* 2012, *51*, 4204−4208) used nuclear magnetic resonance (NMR) spectroscopy to peer into what might be present at the physiological condition of many bacteria, pH 7. At the onset, this seemed like a straightforward characterization, but the results turned out to be surprising.

The spectral complexity implied that the proposed equilibrium was actually not the case, and additional DPD-

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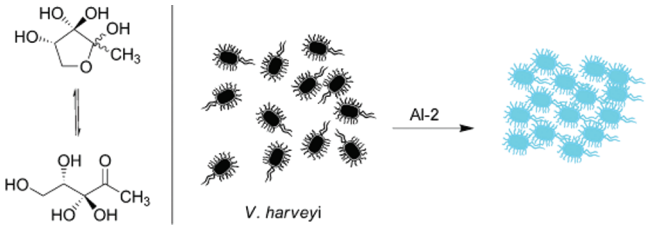

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derived signaling molecules might actually be produced at a neutral pH. The most likely linear form was an oxygenated form, tetrahydroxypentan-2-one, which then teetered in equilibrium with two cyclic isomers. This was interesting since oxygenated forms have previously been shown to play a role in quorum sensing. One of the cyclic forms of the oxygenated DPD can form a complex with boron during *Vibrio* quorum sensing, and a phosphorylation event of DPD has been shown to alter gene expression in enteric bacteria such as *Salmonella* and *E. coli*. The researchers went on to study alkylated forms of DPD that alter the equilibrium between linear and cyclic forms. At neutral pH, the alkylated DPD molecules displayed a ratio of linear to cyclic forms of approximately 1 to 1. Learning more about these equilibria is especially interesting when compared to existing data on quorum sensing in various species. While *Vibrio* sense the cyclic form complexed with boron, species such as the human pathogen *Salmonella* can sense the linear species and alter their cellular program. These data indicate that simple DPD molecules may use isomer switching at physiological pH, and this is likely to regulate downstream biological activity during quorum sensing. **Jason G. Underwood, Ph.D.**

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