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Spotlight

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GENERATING ELECTRICITY AT A SNAIL'S PACE

Traditionally, a biofuel cell refers to system wherein a microbial community or enzyme set is put to work generating electricity. By feeding the system the proper substrates, such as sugars in the case of microbes, a collectable form of energy is the result. A new study by Hálamková *et al.* (*J. Am. Chem. Soc.*, 2012, *134*, 5040−5043) takes the next step by fueling energy production with the circulating glucose inside of a living organism, a terrestrial snail.

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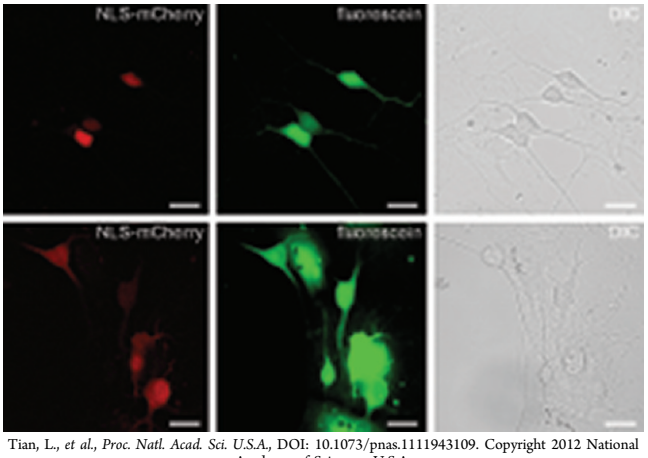
The concept of a fuel cell inside of a living organism has been around for decades, and this general idea is of great interest for powering devices used in medical or surveillance purposes. To bring this concept to reality, a type of *in vivo* battery was constructed with two enzymes paired for concerted oxidation and reduction. The serious hurdle to the system was how to link the two enzymes in such a way that direct and efficient electron transfer could take place. To achieve this, the researchers constructed the cathode and anode with a type of nanostructure mesh made up of carbon nanotubes, which were then functionalized with a cross-linker compound to immobilize enzymes to the surface. For the cathode reaction, the oxygen-reducing enzyme laccase was utilized. At the anode, a pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase was immobilized. Unlike many enzymes that could transfer an electron, this enzyme does not require exogenous cofactors or generate undesirable products such as peroxides from oxygen. After testing the electrical properties *in vitro*, the cathode and anode were surgically placed in a snail's main body cavity which is filled with hemolymph, the gastropod's equivalent of blood. Connecting these implanted electrodes to an electrical circuitry outside of the snail allowed monitoring of the voltage and current generated by the internal biofuel cell. Remarkably, the cell produced energy for 30−60 min and could be recharged by allowing the snail to rest, or by feeding the snail. The biofuel cell remained active for months inside of the snail. This study demonstrates that an almost science fiction-like concept that has long remained outside of an organism can be moved into a living, moving organism.

Jason G. Underwood, Ph.D.

ESTER−ESTERASE TEAM FOR CELL-SPECIFIC LABELING

Small molecules are versatile probes for examining and manipulating biological processes. A persistent challenge in the use of these chemical probes is delivering them to specific cell types in the context of more complex biological environments, such as tissues. Tian *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, published online March 12, 2012; DOI: 10.1073/pnas.1111943109) now describe an innovative approach that uses esterified probes and an exogenous esterase to enable delivery to cells with high specificity.



Tian, L., *et al., Proc. Natl. Acad. Sci. U.S.A.,* DOI: 10.1073/pnas.1111943109. Copyright 2012 National Academy of Sciences, U.S.A.

An ester group is often added to a molecular probe to improve its cellular permeability. Upon entering the cell, nonspecific esterases remove the ester moiety and free the active compound. Central to the authors' approach was identifying an ester that was resistant to nonspecific esterases normally present in the cell, yet susceptible to an esterase from another source. Various esters of fluorescein, which are not fluorescent, were screened, and one containing a cyclopropane moiety was found to be resistant to native cellular esterases. Then, of a

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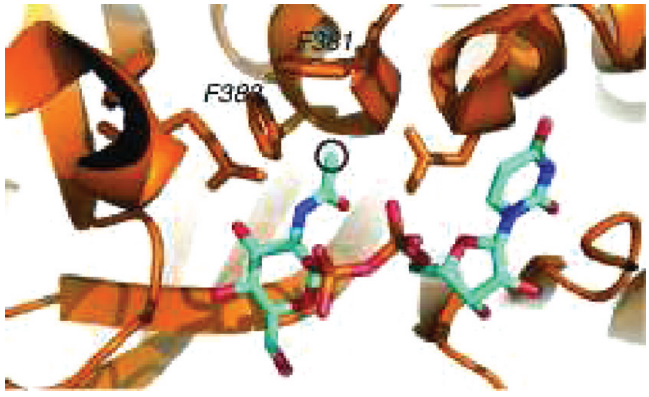
panel of enzymes from a variety of organisms, porcine liver esterase (PLE) was found to be the sole esterase capable of hydrolyzing the cyclopropane-ester-containing fluorescein. To test the validity of this esterase-ester pair, live cells were engineered to produce PLE and then incubated with the ester-modified fluorescein. The cells became fluorescent, indicating that PLE was able to remove the ester group from the fluorescein and restore its fluorescent properties. To demonstrate the utility of this approach, rat neurons were specifically labeled in the presence of other nervous system cells both in culture and in brain tissue. In addition, intracellular communication via gap junctions, which couples the cytoplasm of neighboring cells, could be detected with this strategy, and the mitosis inhibitor monastrol could be selectively activated in PLE-containing cells. This study illustrates how this approach can be generally applied toward the investigation of a wide range of activities in a cell-specific manner.

Eva J. Gordon, Ph.D.

CROSS-LINKING O-LINKED GLYCOSYLATION

Hundreds of proteins in animals and higher plants from diverse structural and functional classes are glycosylated with an O-linked *β*-*N*-acetylglucosamine (O-GlcNAc), but the functions of this posttranslational modification have remained elusive. Some evidence suggests that O-GlcNAc may modulate other processes such as protein binding interactions and phosphorylation events, but a more precise delineation of the functional role of O-GlcNAc is yet to be defined. To this end, Yu *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, published online March 12, 2012; DOI: 10.1073/pnas.1114356109) now report an elegant combination of chemical and genetic methods to pinpoint potential protein binding partners for O-GlcNAc.



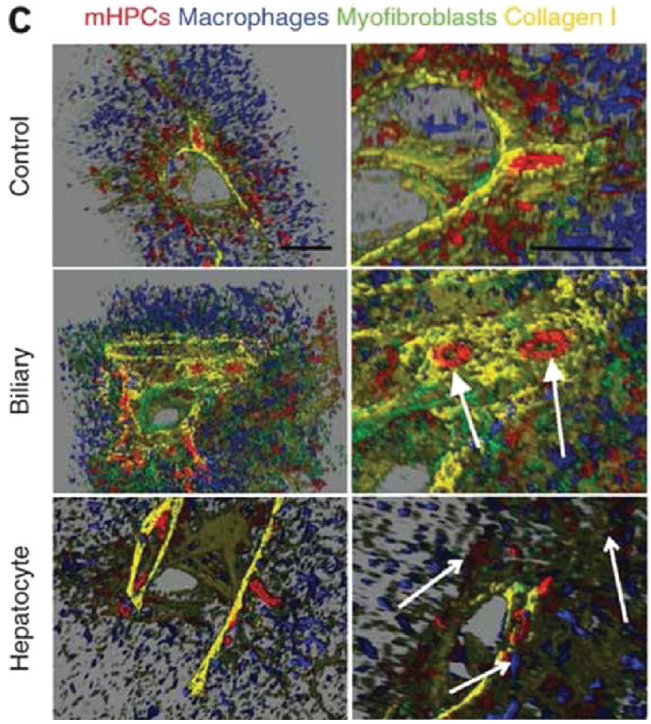
Yu, S.-H., *et al., Proc. Natl. Acad. Sci. U.S.A.*, DOI: 10.1073/pnas.1114356109. Copyright 2012 National Academy of Sciences, U.S.A.

Key to the strategy for finding O-GlcNAc binding partners was the design of a photoactivatable O-GlcNAc analogue containing a diazirine cross-linking group, called O-GlcNDAz. It was first demonstrated that a peptide substrate could be modified with O-GlcNDAz using recombinant O-GlcNAc transferase (OGT). Then, the O-GlcNDAz-containing peptide was incubated with RL2, an antibody that specifically recognizes the O-GlcNAc modification, and irradiated with UV light, resulting in cross-linking of the O-GlcNDAz-modified peptide to the antibody. Next, cells were cleverly engineered to produce UDP-GlcNDAz, which would serve as the substrate for cellular OGT and enable production of proteins containing O-GlcNDAz. Indeed, when lysates of the engineered cells were incubated with RL2 and irradiated with ultraviolet light, RL2 was linked to O-GlcNDAz, suggesting that intracellular proteins were glycosylated with the O-GlcNAc analogue. This set the stage to examine which proteins in live cells might interact with proteins containing O-GlcNDAz. Live engineered cells were irradiated, and nucleoporins, which are normally modified with O-GlcNAc, were examined for evidence of cross-linking. Photo-cross-linked nucleoporin complexes could be detected, and analysis of the complexes revealed several known nuclear transport factors. This approach identified nuclear transport as a potential function for O-GlcNAc modification, and offers a general new strategy for finding binding partners to proteins modified with O-GlcNAc.

Eva J. Gordon, Ph.D.

GIVING NEW LIFE TO THE LIVER

The liver, which has numerous important functions including detoxifying chemicals, metabolizing drugs, and secreting bile to aid digestion, is composed of two main cells types: hepatocytes, which make up the majority of the liver tissue, and cholangiocytes, which are the cells that line the bile-secreting ducts. Chronic liver disease has a variety of causes, including viral infection, excessive alcohol consumption, and certain metabolic or autoimmune disorders. Injury to the liver as a result of these conditions can induce the regeneration of liver cells, but the only cure for end-stage liver disease is transplantation. Thus, a better understanding of the liver regeneration process has significant therapeutic implications. Boulter *et al.* (*Nat. Med.*, published online March 4, 2012; DOI: 10.1038/nm.2667) now explore the specific roles of two key signaling pathways implicated in the liver regeneration process, Notch and Wnt, in the generation of new hepatocytes and cholangiocytes.



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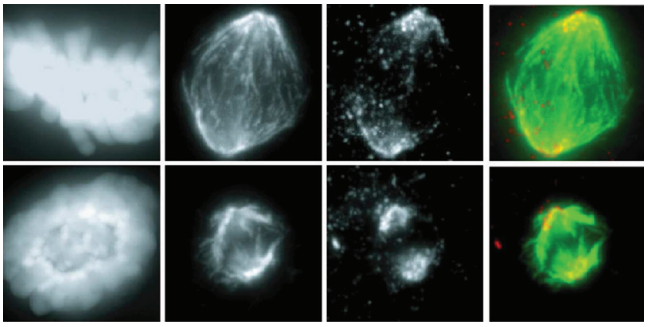
Hepatic progenitor cells (HPC) are bipotent liver precursor cells, which means they can differentiate into either hepatocytes

or cholangiocytes. Using mouse models and tissues from patients with liver disease, the contributions of the Notch and Wnt pathways in determining the fate of HPCs were investigated. Interestingly, it was found that signals from distinct cell types in the surrounding tissue directed cell fate. For example, it was found that during regeneration of the bile ducts, a Notch ligand expressed in myofibroblasts promoted Notch signaling in HPCs that led to cholangiocyte formation. On the other hand, during hepatocyte regeneration, Wnt3a expression in macrophages induced Wnt signaling in HPCs that directed their development into hepatocytes. These insights into the pathways that guide HPC cell fate may lead to new therapeutic strategies for liver regeneration.

Eva J. Gordon, Ph.D.

■ DYNEIN DISRUPTED

Dyneins carry out many heavy lifting tasks in cells, including those that support cell, organelle, and protein movement and the organization of cell division. Because these motor proteins convert ATP into mechanical energy quickly, scientists have not had many tools to study their activity within the cell. Now, a newly discovered class of small molecule inhibitors of dyneins will allow researchers to probe the function of these proteins in greater detail (Firestone *et al.*, *Nature*, published online March 18, 2012; DOI: 10.1038/nature10936).



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Dyneins have important transport functions in immotile primary cilia, sensory organelles found in almost all mammalian cells. Firestone *et al.* discovered these new inhibitors through a high-throughput screen for compounds that block Hedgehog signaling, a primary cilium-dependent process. One Hedgehog pathway inhibitor also perturbed primary cilia structure, which led the researchers to synthesize a set of analogue compounds to better understand the mechanism of action. Several of these 2,4-dichlorobenzoyl dihydroquinazolinone analogues, named ciliobrevins, continued to block Hedgehog signaling and the development of cilia. After short-term exposure of cells to the active compounds, Hedgehog pathway transcription factors and other transport proteins accumulated at the distal tip of primary cilia, suggesting that these compounds might affect cytoplasmic dynein-dependent protein transport. The researchers then tested the effect of ciliobrevins on other known functions of cytoplasmic dynein in living cells. Ciliobrevins prevented mitotic spindle assembly and kinetichore-microtubule attachment during cell division. Ciliobrevins also disrupted normal organelle movement: *Xenopus* melanosomes did not aggregate normally, and peroxisomes in treated *Drosophila* cells were stalled. Studies with dynein-coated glass slides and fluorescently labeled microtubules revealed that ciliobrevins slowed the ATP-dependent movement of microtubules along dyneins, and enzymatic studies confirmed that the compounds inhibit dynein ATPase activity.

A few other small molecules have shown dynein inhibition, but with significant limitations. Two are relatively nonspecific, while another is not active in living cells. These ciliobrevins are the first molecules to show specific activity against dyneins *in vitro* and *in vivo* and provide hints for the development of inhibitors against other similar proteins.

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