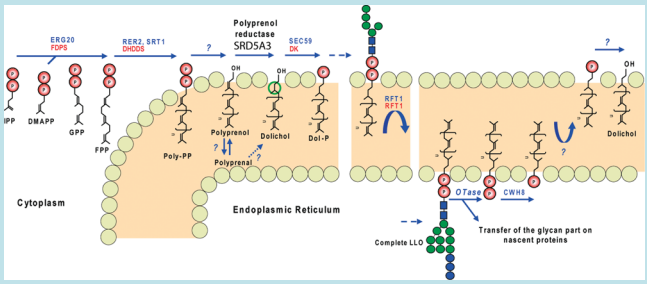
Spotlight

A Lipid Link in N-Glycosylation



Reprinted from *Cell*, *142*, Cantagrel, V., *et al.*, SRD5A3 Is Required for Converting Polyprenol to Dolichol and Is Mutated in a Congenital Glycosylation Disorder, 203–217. Copyright 2010, with permission from Elsevier.

Eukaryotic cells tag particular asparagine residues with N-linked glycans, a process critical for the folding and trafficking of membrane and secreted proteins. This protein modification requires a carrier lipid, dolichol, in the endoplasmic reticulum that shuttles the glycan structure to the asparagine side chain of the receiving protein. Researchers had identified most of the enzymes involved in the synthesis of dolichol from isoprene units, other than a final enzyme that reduces the polyprenol to dolichol. Now, Cantagrel *et al.* (*Cell*, 2010, *142*, 203–217) report an enzyme, steroid 5α-reductase 3 (SRD5A3), that facilitates this reaction. Mutations in this enzyme cause a new type of congenital disorder of glycosylation (CDG), whose symptoms including seizures, blood clotting disorders, and an altered appearance because of abnormal fat distribution or changes in eye structure.

The researchers identified a family with affected individuals and used genome analysis and mapping to discover deletions and insertions in the *SRD5A3* gene. Individuals with the disorder lacked whole glycan chains on specific proteins, suggesting a link to problems with either the synthesis or transfer of these carbohydrates. Because no problems with the glycans were observed, the researchers traced the problem to the synthesis of the lipid linker. Dosing mutant human fibroblasts with dolichol led to N-glycan synthesis at levels that met or exceeded that of healthy control cells. The researchers identified a related enzyme with conserved function in yeast and demonstrated that this enzyme, DFG10, and SRD5A3 converted polyprenol to dolichol.

Because SRD5A3, despite its name, does not act on a steroid, the results suggest a broader set of lipid substrates for this class of enzyme. SRD5A3 is not the only reductase that produces dolichol in cells; human fibroblasts, mouse embryos and yeast cells with mutant SRD5A3 still produced lower levels of dolichol, probably through a different synthetic pathway. The changes in the nervous system, eye structures, skin, and clotting factors in patients with this CDG suggest undiscovered roles for N-glycosylation in a variety of developmental processes. **Sarah A. Webb, Ph.D.**

Parasite Branches Out to Metabolize Carbon

The hundreds of millions of cases of malaria that plague the world's population keep the search for drugs that can effectively combat the disease an imperative one. The identification of new drug targets in *Plasmodium* parasites, the species that causes malaria, is a vital component of drug discovery efforts for malaria treatment, and a detailed understanding of the metabolic pathways utilized by *Plasmodium* parasites will help guide such efforts. For example, knowledge of how the parasites metabolize carbon would elucidate key aspects of the pathways involved in plasmodial mitochondrial electron flow, haem biosynthesis, and protein acetylation (especially at histones), each of which contain potential malarial drug targets. Now, Olszewski *et al.* (*Nature* 2010, *466*, 774–778) report that, in sharp contrast to the tricarboxylic acid cycle in humans, blood-stage *Plasmodium* parasites have devised a branched, linear pathway for metabolizing carbon.

To explore carbon metabolism in the parasites, parasite-infected red blood cells were grown in medium containing isotopically labeled glucose, aspartate, or glutamine. Liquid chromatography–mass spectrometry was then used to monitor the isotopic labeling patterns that emerged over the 2-day parasite cell cycle. It was found that the major carbon sources for the TCA cycle in *Plasmodium* are glutamine and glutamate, contrasting with the TCA cycle in many other organisms in which glucose serves as a primary carbon source. Further, it was determined that mitochondrial carbon metabolism in *Plasmodium* parasites proceeds through two distinct linear branches, an oxidative branch and a reductive branch, that each lead to the production of malate. Intriguingly, further exploration led to the finding that acetyl-coenzyme A generated by the reductive branch of mitochondrial carbon metabolism is used for his-

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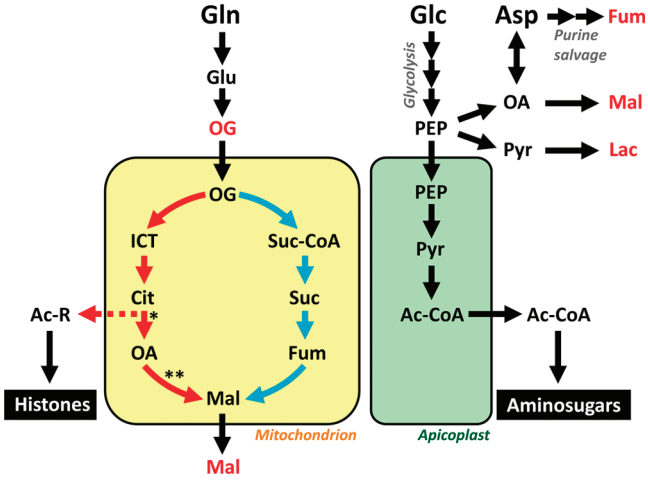

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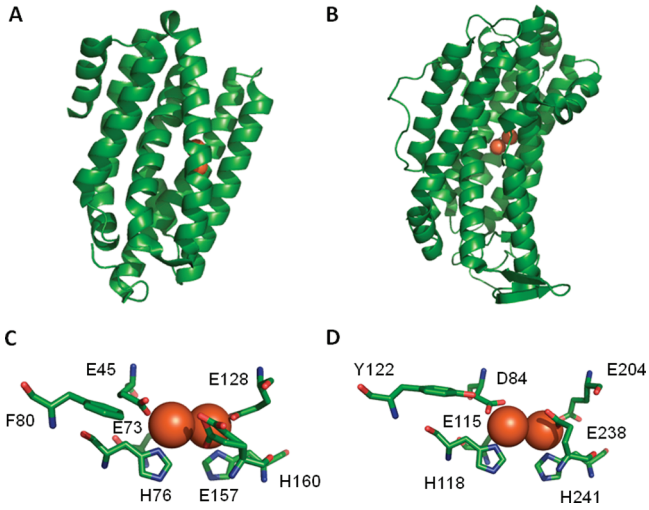
tone acetylation, whereas acetyl-coenzyme A generated from glucose via glycolysis is used for amino sugar acetylation. This model for carbon metabolism in *Plasmodium* illustrates how customized mechanisms can evolve to suit the metabolic needs of organisms that reside in unique environments. **Eva J. Gordon, Ph.D.**

Fueling Alkane Biosynthesis

The substances that fuel our planes, trains, automobiles and the like are largely made up of alkanes, which in turn are largely obtained from fossil fuels. Environmental and other concerns that surround fossil fuel consumption have fueled intense investigation into developing alternative, renewable methods for alkane generation. Diverse organisms naturally produce alkanes, but the biosynthetic pathways involved are for the most part undefined, in eukaryotes as well as in prokaryotic systems. Now, Schirmer *et al.* (*Science* 2010, *329*, 559–562) report the characterization of an alkane biosynthesis pathway in cyanobacteria.

The search for the alkane biosynthetic pathway in cyanobacteria began with the examination of the culture extracts of 11 cyanobacterial strains for the presence of alkanes. While 10 strains produced alkanes, one did not, and subtractive genome analysis led to the identification of two candidate genes, exemplified by open reading frames orf1593 and orf1594 from the *Synechococcus elongatus* strain PCC7942. PCC7942_orf1593 appears to be a member of the short-chain dehydrogenase or reductase family, while PCC7942_orf1594 belongs to the ferritin-like or ribonucleotide reductase-like family. Coexpression of various PCC7942_orf1593 and PCC7942_orf1594 orthologs in *Escherichia coli*, which does not produce alkanes, resulted in the production of several alkanes, predominantly pentadecane and heptadecene. Furthermore, removal of PCC7942_orf1593 and PCC7942_orf1594 in a *Synechocystis*



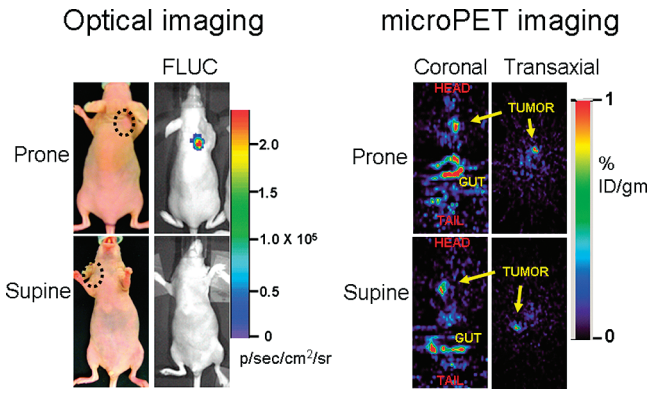
From Schirmer, A., *et al.*, *Science*, 2010, *329*, 559. Reprinted with permission from AAAS.

strain resulted in the disappearance of alkanes in the extracts. The results indicate that PCC7942_orf1593 and PCC7942_orf1594 are both necessary and sufficient for alkane biosynthesis. Examination of the biosynthetic mechanism of alkane generation suggested that PCC7942_orf1593 reduces acyl–acyl carrier protein molecules to their corresponding fatty aldehydes, and PCC7942_orf1594 then catalyzes the decarbonylation of the fatty aldehydes to yield the corresponding alkanes. These insights into microbial alkane production are an exciting contribution to the development of innovative new strategies for generating renewable fuels. **Eva J. Gordon, Ph.D.**

A PET Approach for Imaging Protein–Protein Interactions

Protein–protein interactions (PPIs) are an integral part of most cellular processes, orchestrating diverse activities ranging from signal transduction to cell division to the mounting of an immune response. The ability to image these interactions in live animals offers a unique and powerful view into their character and dynamics and facilitates our ability to manipulate them for exploratory and therapeutic applications. However, a significant challenge associated with this goal involves the capacity of the imaging method to effectively and quantitatively track the often transient and low level PPIs that exist within the context of a live animal. Now, Massoud *et al.* (*Nat. Med.* 2010, *16*, 921–926) tackle this challenge by combining the high sensitivity and noninvasive nature of the imaging method positron emission tomography (PET) with the PPI-reporting capabilities of a protein-fragment complementation assay (PCA).

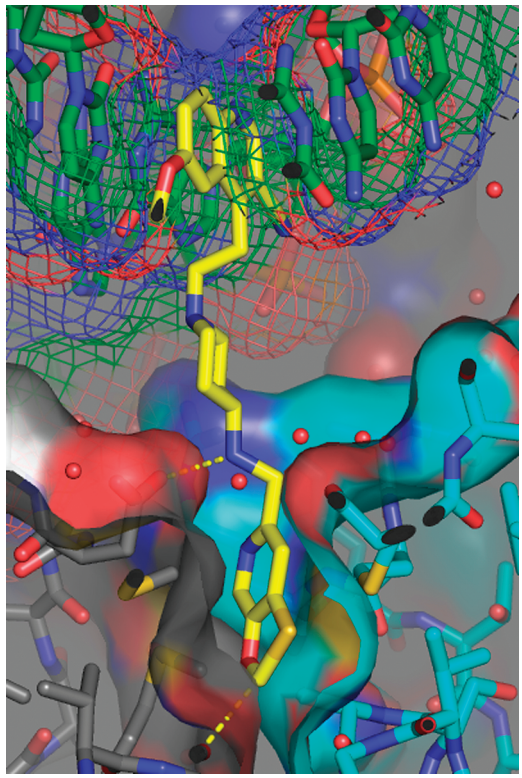
For the PCA, herpes simplex virus type 1 thymidine kinase was used as the reporter protein, and its substrate, which to be active



in PET requires the incorporation of a positron-emitting radioisotope such as ^{18}F-fluorine, was an ^{18}F-containing guanine derivative referred to as FHBG. The thymidine kinase was strategically split between threonine 265 and alanine 266, to become activated only upon the interaction of two proteins, each of which was fused to one of the thymidine kinase fragments. Notably, a point mutation was introduced that significantly enhanced PPI-induced complementation. The utility of this approach was demonstrated with three distinct PPIs: the FKBP12-rapamycin-binding domain and FKBP12 in the presence of rapamycin; hypoxia-inducible factor 1α and von Hippel-Lindau tumor suppressor; and the intramolecular folding of estrogen receptor ligand-binding domains in the presence of appropriate estrogen receptor ligands. When xenografts were created in mice using cells expressing the FKBP-based thymidine kinase reporter system, the PPI occurring within the tumor in the presence of rapamycin could clearly be imaged with PET. Moreover, imaging the tumors using this system was clearly superior over imaging analogous xenografts designed for optical imaging methods. This exciting approach can potentially be expanded to the imaging of other PPIs in various animal models and for a variety of therapeutic applications. **Eva J. Gordon, Ph.D.**

New Drugs for Resistant Targets

In order to develop new medicines that are effective against drug-resistant bacterial strains, it is critically important to understand the mechanism of action of the drug as well as the mechanisms by which the bacteria acquire drug resistance. Bacterial type IIA topoisomerases such as DNA gyrase are important antibacterial drug targets that are effectively inhibited by fluoroquinolones, several of which are in clinical use. However, the recent emergence of fluoroquinolone-resistant strains demands the development of new agents that can more effectively attack these important targets. Now, Bax *et al.* (*Nature* 2010, *466*, 935−940) report the crystal structure of DNA gyrase in complex with DNA and a member of a new family of small molecule DNA gyrase inhibitors, illuminating key mechanistic details surrounding compounds that interact with this target.



In comparing the crystal structures of DNA gyrase in complex with a 20 mer DNA duplex and either a novel bacterial topoisomerase inhibitor referred to as GSK299423 or a fluoroquinolone, numerous key differences were observed. First, though GSK299423 was found to bind near the fluoroquinolone binding site, the fluoroquinolone binds to a protein−DNA complex in which the doubled-stranded DNA has already been cleaved, while GSK299423 interacts with the complex prior to DNA cleavage. In addition, fluoroquinolones appear to interact principally with the cleaved DNA, stabilizing it and preventing the enzyme from religating it, while GSK299423 interacts with both protein and DNA, effectively forming a bridge between the two. The structures also reveal the presence of a single Mn^{2+} ion in the active site, though the precise location differs slightly depending on which inhibitor is bound. Regardless, the environment surrounding the metal alludes to an intriguing and potentially critical role in the DNA cleavage reaction. These insights into the mechanism of action of type IIA topoisomerase inhibitors offer a unique view into topoisomerase function, and will likely facilitate the design of new antibiotics effective against certain drug-resistant bacterial strains. **Eva J. Gordon, Ph.D.**