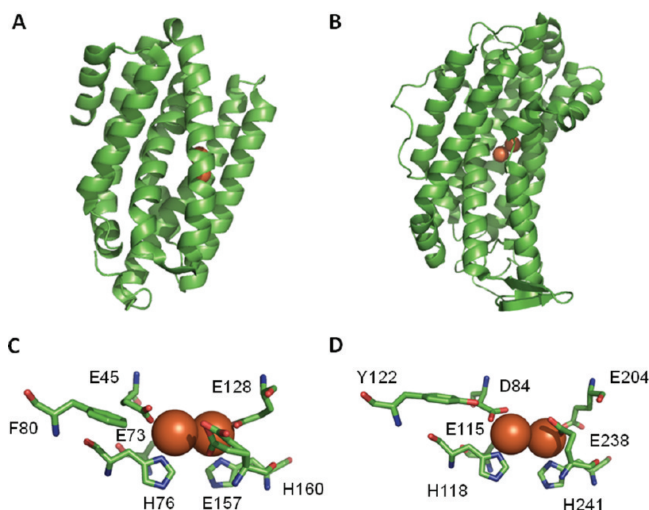
Spotlight

Best of Chemical Biology 2010

2010 was an exciting year for chemical biology, and we've used our Spotlight section to highlight new developments every month. Now, looking back, the Editors have compiled a list of articles representing some of the most interesting and relevant research from all of 2010. As with our monthly Spotlights, it is simply not possible to recognize all of the amazing work being published, and this list is not meant to be comprehensive. However, we hope that this feature will at least provide a sense of the richness and quality of research being published in chemical biology as we move forward into a new decade. Happy New Year!

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.



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

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 elongates* 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* 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.

Life from a Digital File

The invention in the early 1980s of machines that could chemically synthesize DNA oligonucleotides in an automated fashion, coupled with revolutionary advances in DNA sequencing that have enabled the rapid and affordable decoding of the entire genomes of numerous organisms, has paved the way for an explosion of applications in fields such as molecular biology and genetic engineering. Surrounding these tremendous advances in our understanding of genomics over the past few decades, one question at the forefront is can we create a living, functioning organism using entirely synthetic DNA? Gibson *et al.* (*Science*, 2010, *329*, 52−56) report the creation of a viable bacterial cell under the control of a genome made up exclusively of synthetic DNA.

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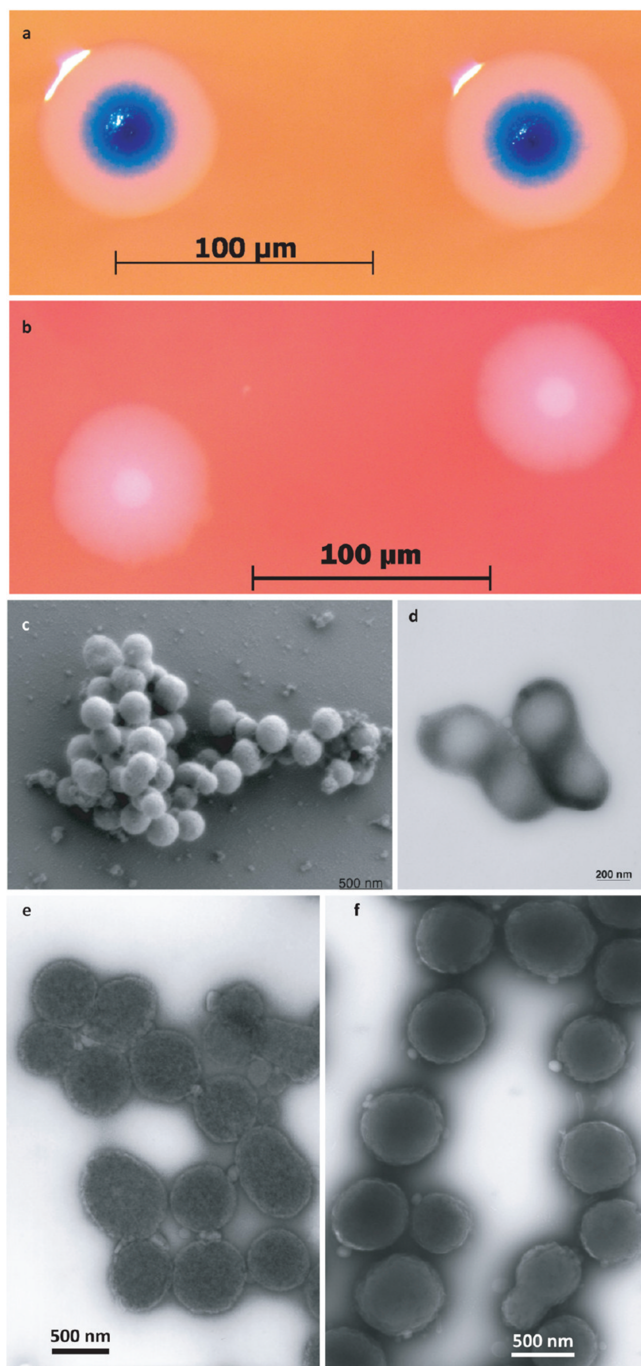
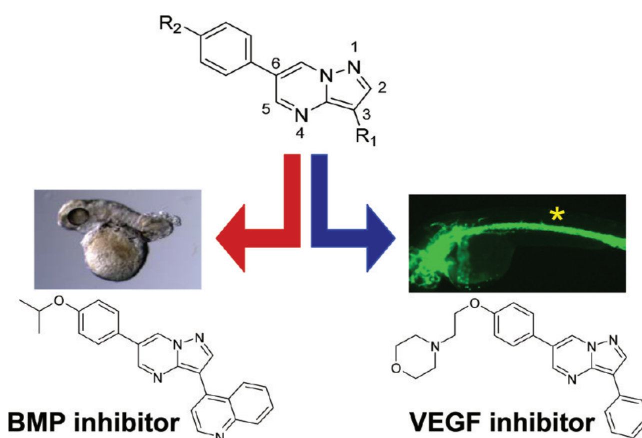


From Gibson, D. G., *et al.*, *Science*, 2010, *329*, 52. Reprinted with permission from AAAS.

The relatively small complement of genes required for independent growth in cell culture made certain species of mycoplasma bacteria an appealing choice for the transplantation and expression of a synthetic genome. The genome from *Mycoplasma mycoides* was selected as the donor genome, and *Mycoplasma capricolum* was chosen as the recipient host cell. Beginning just with the approximately 1 million base-pair *M. mycoides* genome sequence in digitized form, its reconstruction using synthetic DNA was undertaken. First, 1078 1-kb DNA cassettes were synthesized. These cassettes were then assembled into 111 10-kb synthetic intermediates, which were subsequently assembled into 11 100-kb synthetic intermediates, which were finally assembled into the complete genome. Importantly, the synthetic DNA contained watermark sequences in four distinct locations so that it could be clearly distinguished from the natural genome. Once the assembly process was complete, the genome was transplanted into a *M. capricolum* host cell whose own DNA had been removed. Remarkably, cells with the transplanted DNA formed colonies with the expected phenotypic properties. This achievement is an exciting proof of principle that designed genomes can be synthesized and inserted into cells to create viable organisms.

Zebrafish: A New Model for Drug Development

A typical scheme for drug discovery and optimization is arduous and sequentially involves biochemical and cell-based studies, animal models, and human trials. Drug candidates failing at the latter stages of testing are financially costly. As a result, *in vivo* screening of compounds at the start of the process is a useful development. To this end, Hao *et al.* (*ACS Chem. Biol.* 2010, *5*, 245−253) successfully demonstrate that the zebrafish model is efficient in the large-scale and rapid assessment of lead compounds.

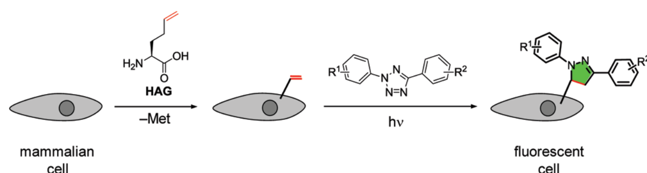


Dorsomorphin was the first reported inhibitor of bone morphogenetic protein (BMP) signaling. Using the zebrafish model, the authors show that this compound has deleterious off-target effects on vascular endothelial growth factor (VEGF) and angiogenesis. An *in vivo* selection strategy using live zebrafish embryos identified three

dorsomorphin analogues with improved specificity toward BMP inhibition and decreased off-target effects. Another analogue showed improved specificity toward VEGF inhibition with minimal effects on BMP. Thus, the zebrafish model provides a novel platform for a rapid and efficient means to probe multiple pathways on a whole organism level and will expedite the identification of drugs with a greater chance of success in human trials.

New Tool for Imaging Proteins in Live Mammalian Cells

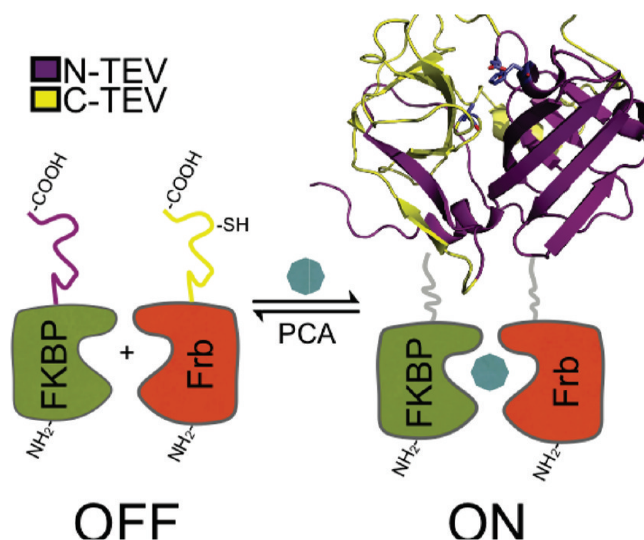
Green fluorescent protein (GFP) is a well-documented protein reporter in cells. However, the large molecular size of GFP can disrupt folding, localization, and function of proteins. More recently, focus has shifted toward the use of small organic groups for studying the spatial distribution of proteins in a cell. Song *et al.* (*ACS Chem. Biol.* 2010, *5*, 875−885) report the development of the first metabolic alkene reporter, homoallylglycine (HAG), for imaging newly synthesized proteins in live mammalian cells.



The authors used structurally tolerant methionine-tRNA to cotranslationally incorporate the unnatural amino acid, HAG. Significant incorporation of HAG was observed in recombinantly expressed proteins and in newly synthesized proteins in mammalian cells. Importantly, HAG-labeling did not perturb protein function. Using "photoclick chemistry", HAG is bioconjugated to a fluorescent form with tetrazole reagents. Time-resolved fluorescent labeling of proteins studies permitted imaging newly synthesized proteins in live mammalian cells with spatiotemporal control. This novel alkene reporter offers an exciting alternative for visualizing proteins and is likely to find broad application leading to new biological insights.

SNIPers and Executioners

The executioner caspases are the final caspases activated during apoptosis, or programmed cell death. These activation events lead to the cleavage of nearly 1000 proteins, a process that propels the cell along its journey to the grave. However, deciphering the specific roles of each executioner caspase, caspase-3, -6, and -7, during the complex process of apoptosis is a formidable challenge. Now, Gray *et al.* (*Cell* 2010, *142*, 637−646) present the design of engineered executioner caspases under the control of a small-molecule activator and the use of this system to interrogate the individual functions of these important enzymes.



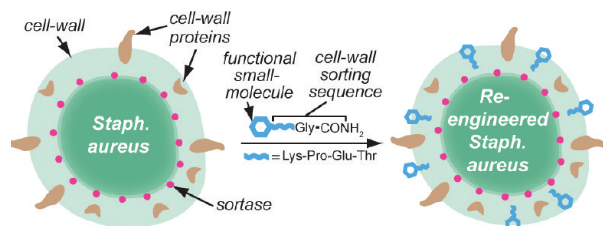
Reprinted from *Cell, 142*, Gray, D. C., *et al.*, Activation of Specific Apoptotic Caspases with an Engineered Small-Molecule-Activated Protease, 637−646, Copyright 2010, with permission from Elsevier.

The design of the engineered caspase system relies on the activity of the NIa tobacco etch virus (TEV) protease and the expression of executioner caspases engineered to contain TEV cleavage sites. Building on a common method used for protein complementation assays, the TEV protease was split, with one portion attached to the protein FKBP and the other attached to FRB. In the presence of rapamycin, FKBP binds FRB, thus bringing the two portions of TEV together and reconstituting the active protease, a construct referred to as the SNIPer. When the engineered versions of caspase-3 or caspase-7 were expressed in a human kidney cell line expressing SNIPer and rapamycin was added to the cells, SNIPer promptly cleaved and activated the engineered caspase, and the cells rapidly underwent apoptosis. Remarkably, it was also found that the inclusion of proteasome inhibitors worked synergistically with caspase activation to promote apoptosis. Interestingly, however, when the engineered version of caspase-6 was activated, the cells underwent apoptosis only when proteasome inhibitors were also present. The authors propose an intriguing model of negative proteolytic regulation, in which the proteasome attempts to restrict caspase activity while caspases endeavor to dismantle the proteasome. This enticing link between the caspase and proteasome activity alludes to the possible therapeutic benefit of combined apoptosis activators and proteasome inhibitors.

Re-engineering Gram-Positive Bacterial Cell Walls

Under pressure from the emergence of drug-resistant bacteria, researchers often focus their efforts on the development of novel drugs. However, Nelson *et al.* (*ACS Chem. Biol.* 2010, DOI: 10.1021/

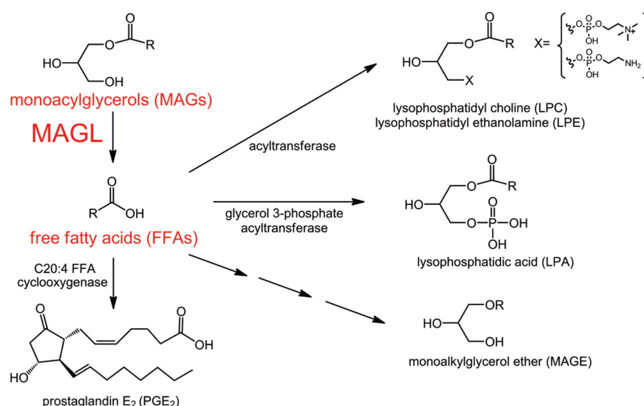
cb100195d) have developed an innovative strategy to combat drug resistance in Gram-positive bacteria. Their method involves "tricking" *Staphylococcus aureus* into incorporating non-natural compounds into its cell wall, thereby making the organism more susceptible to therapeutics. The novel approach presents an opportunity to affect the way bacteria interact with host cells.



The authors employ the endogenous enzyme sortase A (SrtA), which incorporates proteins into the bacterial cell wall. Typically, SrtA recognizes its substrate *via* a conserved pentapeptide motif, LPETG, proximate to the C-terminus of the secreted protein. Upon substrate recognition, SrtA forms an acyl-enzyme intermediate by cleaving the threonine−glycine bond of the pentapeptide motif. This event is followed by an attack of the peptidoglycan precursor (lipid II) and the formation of an adduct that is eventually incorporated into the bacterial cell wall. Using these properties of SrtA, exogenous small molecules varying in functionality, such as fluorescein, azides, and biotin, were incorporated into the *S. aureus* cell wall. A wide array of experimental techniques such as epifluorescence and electron microscopy, flow cytometry, mass spectrometry, and biochemical cell wall extraction were used to confirm the covalent incorporation of the non-native small molecules into the peptidoglycan layer. This report presents the first example of cell wall engineering of any pathogenic Gram-positive bacteria not involving the genetic manipulation of the organism. Given the global prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and related nosocomial infections, the importance of new approaches to combat drug-resistant bacteria cannot be overstated. This unique method offers fresh impetus to efforts to develop new antimicrobial tools.

A Lipid Gatekeeper in Cancer Cells

A variety of chemical and behavioral changes distinguish cancer cells from their normal counterparts. As one emerging example, malignancy has been linked with heightened lipid levels, both through increased production by fatty acid synthase and release from their cellular storehouses. However, researchers hadn't understood how these biochemical changes were connected to the disease process. Now, Nomura *et al.* (*Cell* 2010, *140*, 49−61) reveal how cancers can hijack a lipid hydrolysis enzyme to promote cancer progression.



Reprinted from *Cell*, *140*, Nomura, D. K., *et al.*, Monoacylglycerol Lipase Regulates a Fatty Acid Network that Promotes Cancer Pathogenesis, 49−61, Copyright 2010, with permission from Elsevier.

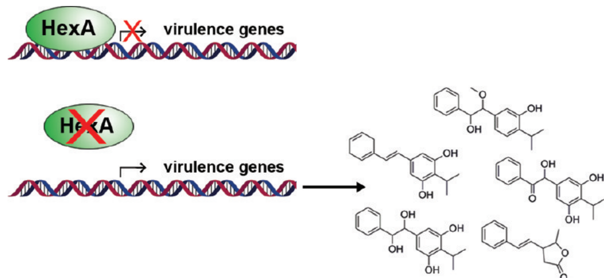
Comparing proteins found in cancer cell lines from a variety of aggressive and nonaggressive ovarian and breast tumors and melanomas, researchers found that the expression of monoacylglycerol lipase (MAGL) was consistently elevated in aggressive cancers. Inhibiting MAGL in aggressive cancer cells significantly reduced their concentrations of free fatty acids (FFAs). The researchers confirmed these results in high-grade primary ovarian tumors. In normal tissues MAGL typically regulates the concentration of monoacylglycerols rather than FFAs, so these results point to a new and conserved role for MAGL in aggressive cancer cells.

Nomura *et al.* then demonstrated the central importance of MAGL in aggressive tumor cell lines. Disruption of MAGL activity in these cell types inhibited cell migration, invasiveness, and survival. Adding FFAs back into the cultures of inhibited cells restored their pathogenicity, indicating that MAGL supports more aggressive cancers by elevating FFAs. These results suggest a biochemical process where obesity or a high fat diet could support malignancy even if the cells do not show increased lipid hydrolysis. In addition, within these cells, MAGL controls the production of downstream agents that boost malignancy such as lysophosphatidic acid and prostaglandins. MAGL sits at an intersection point for a variety of lipid signals connected to cancer's origins and could prove to be an important new target for cancer therapies.

Maximizing Metabolites

Secondary metabolites are rich sources of structurally diverse, biologically active compounds, many of which are valuable biological probes or have intriguing therapeutic potential. However, there is a large disconnect between the number of known bacterial secondary metabolites and the number of biosynthetic genes within bacterial genomes capable of producing such compounds. In an effort to bridge this gap, Kontnik *et al.* (*ACS Chem. Biol.* 2010, *5*, 659−665) explore the downstream regulation of metabolite production in *Photorhabdus luminescens*, a Gamma proteobacteria whose second-

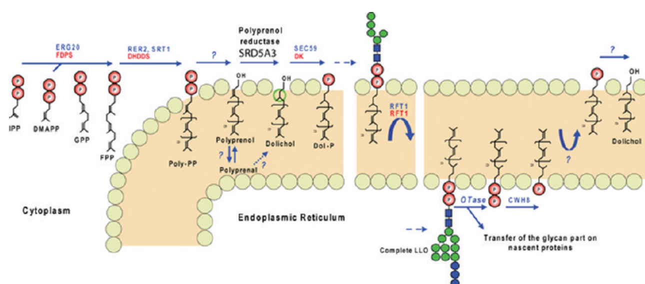
ary metabolite production is a vital component of its symbiotic relationship with nematodes and insect larvae.



Metabolomic profiling of a strain *P. luminescens* in which the gene for the transcriptional regulator HexA had been knocked-out showed a dramatic increase in concentration of numerous metabolites, including various stilbene derivatives. This approach sheds light onto the regulatory control of secondary metabolite generation and points to a promising strategy for novel small molecule discovery.

A Lipid Link in N-Glycosylation

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), which 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.



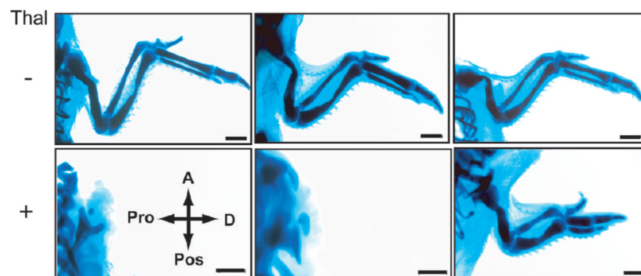
Reprinted from *Cell*, *142*, Cantagrel, V. *et al.*, *SRD5A3* Is Required for Converting Polyprenol to Dolichol and Is Mutated in a Congenital Glycosylation Disorder, 203−217, Copyright 2010, with permission from Elsevier.

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.

Thalidomide Makes a Molecular Debut

In the 1950s and 1960s, many pregnant women battled morning sickness with what they thought was a sedative drug, thalidomide. Sadly, the unseen effects of thalidomide on the fetus would not be fully realized until over 10,000 children were born with birth defects, mostly involving limb formation or size abnormalities. Decades later, the molecular mechanism of the drug's ill effects remained controversial and largely unsolved.



From Ito, T, *et al.*, *Science*, 2010, *327*, 1345. Reprinted with permission from AAAS.

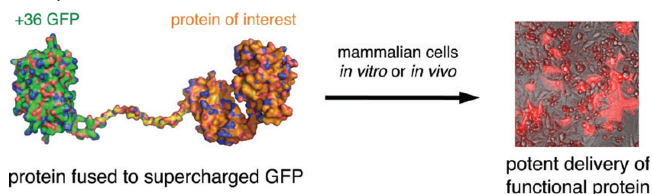
Now, a new study by Ito *et al.* (*Science* 2010 *327*, 1345−1350) has used a powerful combination of biochemistry and developmental biology to show the first bona fide direct target of thalidomide. Using special beads coupled with a thalidomide derivative, the researchers fished out two proteins from mammalian cell extracts that specifically bound to the drug. The candidate proteins, CRBN and DDB1, both bound to the beads, but further tests proved that CRBN was the directly associated target. Luckily, the DDB1 not only came along for the ride by binding to CRBN but also gave a clue to one possible effect of thalidomide. DDB1 was previously found to be as-

sociated with a protein complex that plays a key role in protein turnover by ubiquitination, a mark that sends proteins to the proteasome for degradation. When the researchers tested the associated protein, CRBN, they found that it was a component of an E3 ubiquitin ligase complex and CRBN also displayed ubiquitination. Moreover, addition of thalidomide caused specific inhibition of ubiquitination.

After localizing the thalidomide-binding region of CRBN, a valuable mutant of the protein was constructed. The YW/AA mutant could still interact with DDB1 and function as an E3 ligase but could no longer bind to thalidomide. Armed with this mutant, two highly divergent models systems of refined embryology, the zebrafish and chicken, were both employed to show that CRBN is the target of thalidomide. Knockdown of zebrafish CRBN protein caused a similar phenotype to thalidomide treatment, a loss of proper fin formation. Protection from the drug was not observed by expression of the wild-type (WT) CRBN but was strikingly apparent with the thalidomide-immune YW/AA rescue in both fish fins and chick limbs. Further experiments demonstrated that FGF proteins could be the main downstream targets of the CRBN/DDB1 complex. These results bring a half-century old mystery into a new molecular limelight but unlock a new mystery to tackle. Since CRBN is present in most known cell types, why are the limbs the main target of this devastating drug?

Protein Delivery: It is Positively the Charge

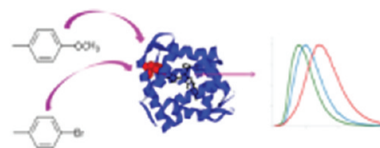
Biomolecules of all sizes can be powerful tools for understanding biology and can also have diverse therapeutic applications. However, while many small molecules can penetrate the cell membrane unimpeded to exert their desired effect inside the cell, proteins typically cannot. Some methods have been devised to facilitate protein entry into cells, but they are generally inefficient, especially for delivery in an *in vivo* setting. Cronican *et al.* (*ACS Chem. Biol.* 2010, *5*, 747−752) now report a novel method for delivering proteins into mammalian cells using a "supercharged" variant of green fluorescent protein (GFP).



The method relies on the generation of a fusion protein comprised of the protein of interest and a GFP containing a net positive charge of 36 (+36 GFP). Proteins fused to +36 GFP entered the cell and could access the cytosol, as was demonstrated with ubiquitin, and the nucleus, as was the case for a recombinase enzyme. Furthermore, when injected *in vivo* into the subretinal space of mice, the recombinase fusion protein was delivered to the mouse retinae. This versatile approach offers a promising new method for the delivery of exogenous proteins into cells for numerous applications.

A Non-natural Bioluminescent Reporter

The incorporation of non-natural amino acids is a powerful approach to alter the properties of proteins. Currently, biochemists incorporate residues with novel functional groups *in vivo* using a combination of altered tRNA/tRNA synthetase pairs and amber codon suppression. Rowe *et al.* (*ACS Chem. Biol.* 2010, *5*, 455−460) exploit this tool to incorporate different non-natural amino acids which alter the bioluminescence of a protein, providing researchers with a new tool for imaging *in situ* and studying protein−protein interactions *in vivo*.

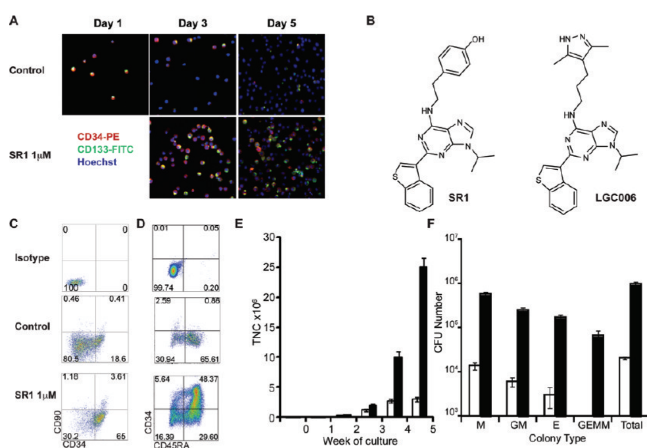


Aequorin contains coelenterazine, a choromophore that bioluminesces when the protein binds calcium. Using orthogonal tRNA/tRNA synthetases and amber codon suppression, four non-natural phenylalanine derivatives were incorporated to replace a noncatalytic tyrosine residue in aequorin. The change in this residue to a non-natural amino acid resulted in a shift in the emission maxima of the protein. To increase the bioluminescence properties, the four non-natural aequorin derivatives were further altered with coelenterazine analogues. The combination of non-natural amino acids and chromophore analogues resulted in a significant shift (up to 44 nm) in the bioluminescence emission wavelength of aequorin. Importantly, the bioluminescence intensity of the non-natural aequorins was high enough to find utility in cell-based assays. This is the first reported spectral shifting of a bioluminescent protein, and this tool is likely to have significant utility in future research.

Out for Blood Cells

Stem cell transplantation provides a disease-free supply of blood cells to help the body fight a variety of hematological disorders and cancers, such as certain anemias, leukemias, and lymphomas. Hematopoietic stem cells (HSCs), which can differentiate into all blood cell types, reside in the bone marrow and to some extent in the peripheral blood, but gaining access to adequate quantities of these cells for transplantation can be a significant challenge. Treatment with the growth factor cytokine granulocyte colony-stimulating factor (G-CSF) is a common method for mobilizing hematopoietic stem and progenitor cells (HSPCs) from bone marrow to peripheral blood, where they can be collected. However, this process is not always

successful, and the pathways involved are not completely understood. The expansion of HSCs in cell culture is a complementary and promising approach for obtaining a sufficient supply of HSCs, but culture conditions that support HSC expansion are not well-defined. Ryan *et al.* (*Nat. Med.* 2010, *16*, 1141−1146) and Boitano *et al.* (*Science* 2010, *329*, 1345−1348) reveal exciting insights into the mechanisms underlying HSC mobilization and expansion.



Reprinted by permission from Macmillan Publishers Ltd.: Nat. Med., Ryan *et al.*, 16, 1141-1146, copyright 2010. From Boitano, A. E., *et al.*, *Science*, 2010, *329*, 1345. Reprinted with permission from AAAS.

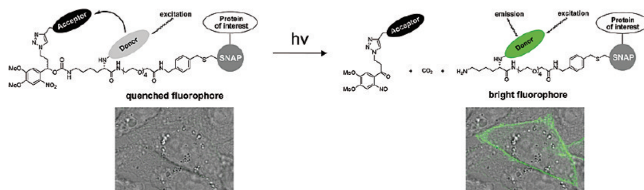
To explore the mechanisms involved in G-CSF-dependent mobilization of HSCs, Ryan *et al.* employed a forward genetic approach in mice. Specifically, use of a congenic mouse model facilitated identification of a 5 MBp region of chromosome 11 that conferred enhanced mobilization of HSPCs, and gene chip expression analysis and quantitative real-time RT-PCR experiments revealed that the enhanced mobilization correlated with a reduction in *Egfr* gene expression in hematopoietic progenitor cells (HPCs) isolated from bone marrow. Further, increased mobilization was observed in mice that either possessed a genetic mutation that reduced EGFR activity or that were treated with erlotinib, a small molecule inhibitor of EGFR activity. Investigation into the mechanism of this effect revealed the involvement of Cdc42, a downstream target of EGFR known to be involved in the migration and adhesion of HSPCs. Specifically, genetic reduction in Cdc42 activity also resulted in enhanced mobilization, suggesting that the mobilization process is controlled in part by EGFR signaling through Cdc42. These findings point to EGFR inhibitors as potential agents for enhancing HSPC mobilization for stem cell transplantation in the clinic.

Under defined cell culture conditions, HSCs can be coaxed to proliferate, which offers another potential source of HSCs for transplantation. However, the proliferation is accompanied by differentiation, which results in a loss of the precious multipotent potential of the cells. In an effort to find conditions that promote expansion without differentiation, Boitano *et al.* used primary human HSCs and confocal microscopy to screen 100,000 heterocycles for compounds that increased the number of cells expressing CD34 and CD133, cell surface markers present on HSCs and HPCs that are lost upon differentiation. A purine derivative referred to as SR1 was found to promote an increase in the number of $CD34^+$ and $CD133^+$ cells. Engraftment studies, which measure the ability of the transplanted cells to generate new blood cells, using immunodeficient mice demonstrated that culture with SR1 leads to substantially more HSCs capable of differentiating into multiple blood cell types compared to uncultured $CD34^+$ cells. Transcriptional profiling experiments pointed to the aryl hydrocarbon receptor (AHR) transcription factor as a likely target for SR1. Indeed, further investigation revealed that SR1 inhibits AHR through a direct binding interaction.

These studies probing the mechanisms that dictate HSC mobilization and proliferation have elucidated two exciting new approaches for increasing access to HSCs for stem cell transplantation, and highlight the power of chemical biological approaches for exploring hematopoietic stem cell biology.

Protein Probes Light the Way

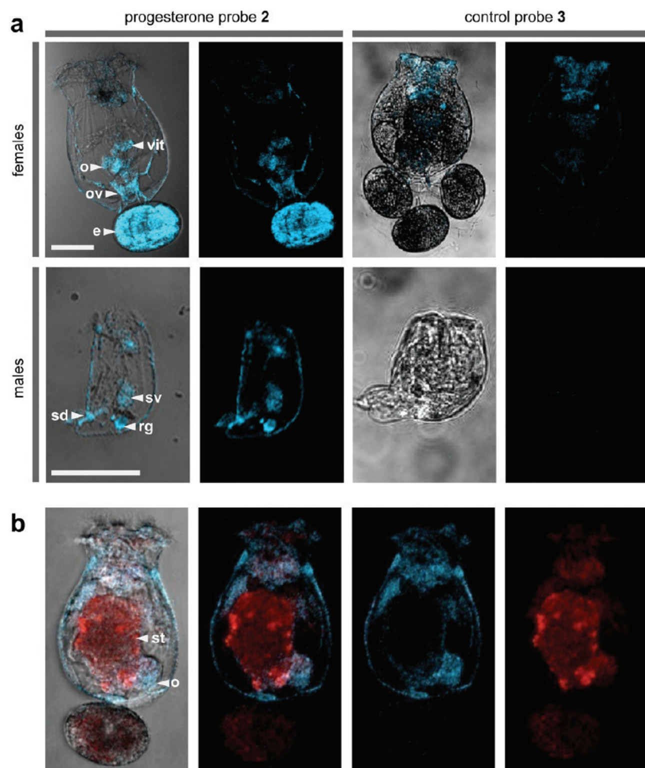
The use of fluorescence in the investigation of protein function has illuminated countless insights into molecular and cellular processes, and innovative advances in fluorescence technology along with protein engineering techniques continue to position this powerful approach at the cutting edge of research. Toward expanding the versatility of fluorescent tools available for exploring biological processes with ever-increasing temporal and spatial resolution, Maurel *et al.* (*ACS Chem. Biol.* 2010, *5*, 507−516) describe a general method for creating proteins labeled with photosensitive probes inside live cells.



The strategy is based on the use of a probe composed of a fluorophore connected to a fluorescent quencher *via* a photocleavable linker. Using SNAP-tag technology, which enables the generation of fusion proteins that can be chemically tagged in live cells, this probe can be chemically attached to a protein of interest, generating a labeled protein that can be rendered fluorescent upon exposure to light. The utility of the approach was demonstrated by characterizing the mobility of a G protein coupled receptor and a lipid-linked cell surface protein in live cells.

Sex and Progesterone in Invertebrates

Not much is known about the sex life of monogonont rotifers, which are submillimeter-sized, nonarthropod invertebrates that generally reside in freshwater environments. However, it is known that these tiny animals can reproduce both sexually and asexually, and recent evidence has implicated the steroid hormone system in the switch from asexual to sexual reproduction. Now, using an impressive suite of chemical and biological methods, Stout *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2010, *107*, 11859−11864) reveal the presence of progesterone and a progesterone receptor in the monogonont rotifer *Brachionus manjavacas*.



Stout, E. P., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *107*, 11859−11864. Copyright 2010 National Academy of Sciences, U.S.A.

Examination of the *B. manjavacas* genome led to the identification of a gene sequence with homology to numerous membrane-associated progesterone receptors. To help find and characterize this receptor in the rotifer, two progesterone-based molecular probes were designed and synthesized. First, use of a fluorescent progesterone derivative demonstrated the presence of a progesterone binding molecule in the reproductive organs of both male and female rotifers. Next, along with an antibody against the fluorescent progesterone, the probe was used to help fish out progesterone-binding proteins from crude rotifer lysates. Immunoprecipitation experiments with the fluorescent progesterone probe, as well as use of a chemically reactive progesterone-derived probe designed to become covalently attached to the receptor active site, led to the identification of a protein that selectively bound to the progesterone probe. Mass spectrometry analysis confirmed that peptides derived from the protein indeed exhibited strong homology with other progesterone receptors. Finally, RNA interference experiments also confirmed the presence of the putative progesterone receptor, and demonstrated that inhibition of this gene led to a decrease in induction of sexuality in first-generation daughters of female rotifers. These findings strongly implicate progesterone in the regulation of the rotifer reproductive system and also offer clues into the evolutionary history of progesterone and the sex steroid system enjoyed by higher animals.