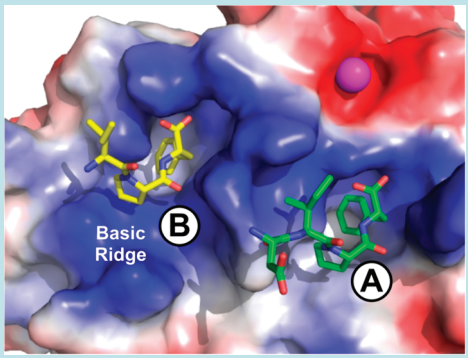
Spotlight

Genome Maintenance Crews



Lu, D., *et al., Proc. Natl. Acad. Sci. U.S.A., 107*, 633–638. Copyright 2010 National Academy of Sciences, U.S.A.

Not unlike other huge, multicomponent conglomerates, genomes require maintenance crews to ensure operations are running smoothly. Genome maintenance is conducted by various enzymes that replicate, recombine, or repair genomic DNA, and bacterial single-stranded DNA-binding proteins (SSBs) protect and stabilize the regions of single-stranded DNA that are generated during these activities. SSBs also function to recruit and sometimes even activate the genome maintenance enzymes, and such SSB activities are essential for bacterial survival. Notably, the highly conserved C-terminal segment through which SSBs bind genome maintenance proteins is absent in eukaryotic SSBs, suggesting bacterial SSBs as intriguing targets for novel antibacterial agents. Toward facilitating further exploration of the role of SSBs in genome maintenance, Lu *et al.* (*Proc. Natl. Acad. Sci.* 2010, *107*, 633–638) report the discovery of four small molecules that inhibit the interaction between bacterial SSB and the genome maintenance protein Exonuclease I (ExoI).

Over 50,000 small molecules were screened using a high-throughput fluorescence polarization assay, leading to the identification of four compounds capable of disrupting SSB binding to ExoI. The compounds were also shown to inhibit SSB-stimulated ExoI activity, but not ExoI activity in the absence of SSB, suggesting that they bind to a site distinct from the ExoI active site. X-ray crystallography studies and kinetic data indicated that three of the compounds directly compete with SSB for binding to ExoI, while the fourth binds to an alternate site on ExoI that leads to allosteric inhibition of the SSB-ExoI interaction. Further investigation revealed interesting subtleties in the specificities of the compounds. For example, one of the compounds appeared to be a specific SSB-ExoI inhibitor, while the others were capable of preventing binding between SSB and two DNA helicases, suggesting their utility as general SSB inhibitors. These compounds are valuable new tools for exploring SSB function and represent an exciting starting point for the generation of novel antibiotics. **Eva J. Gordon, Ph.D.**

Fighting Off FGF-2

Angiogenesis, the formation of new blood vessels, is critical for fundamental processes such as growth, development, and wound healing, but its misregulation has been implicated in numerous diseases including cancer and atherosclerosis. Inhibitors of angiogenesis, many of which target the angiogenic factor vascular endothelial growth factor, have shown much promise in combating unwanted angiogenic processes, but cells can outsmart this approach by upregulating other angiogenic factors such as fibroblast growth factor-2 (FGF-2). Using an impressive combination of peptide array technology, molecular dynamics, nuclear magnetic resonance (NMR) spectroscopy, and in silico, biochemical, and cellular screening methods, Colombo *et al.* (*J. Biol. Chem*, First Published on January 7, 2010, DOI: 10.1074/jbc.M109.085605) now describe their discovery of novel angiogenesis inhibitors targeting FGF-2.

Based on the recent discovery of a new site of interaction between FGF-2 and the endogenous angiogenesis inhibitor thrombospondin-1 (TSP-1), a search began for the FGF-2 binding sequence on TSP-1. Screening of over 200 peptides for their ability to bind FGF-2 revealed a 15-residue sequence referred to as DD15. Molecular dynamics simulations were employed to identify the key components of the DD15–FGF-2 interaction. This information was validated by NMR spectroscopy and used to create a pharmacophore that could be utilized to screen approximately 290,000 compounds in silico. Of 258 compounds identified in the virtual screen, 19 were tested experimentally, and of these 3 were found to competitively inhibit the interaction. The compounds also inhibited the binding of FGF-2 to endothelial cells and FGF-2-induced endothelial cell proliferation, and the most active compound inhibited angiogenesis induced by FGF-2 in an *in vivo* assay. The promising angiogenesis inhibitors discovered in this study highlight the potential of this multidisciplinary approach for the discovery of novel antiangiogenic drugs. **Eva J. Gordon, Ph.D.**

From Cancer to Cognition, An Allosteric Approach

The concept of allosteric regulation, which refers to modulation of protein activity that occurs upon ligand binding at a site other than

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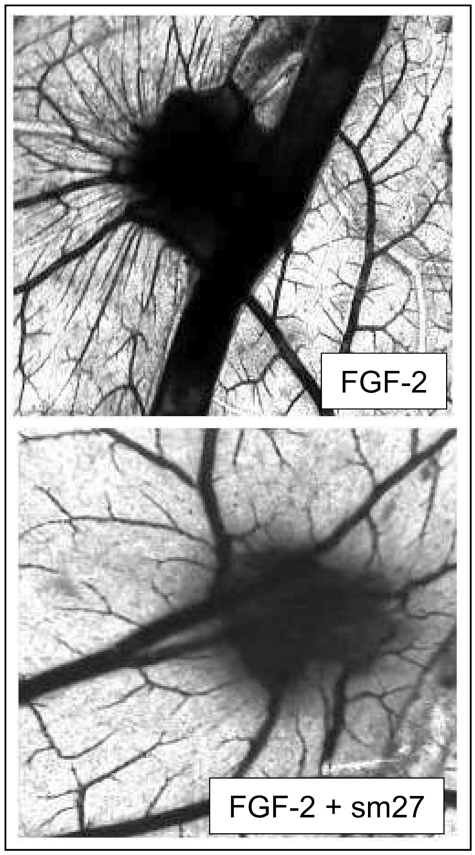
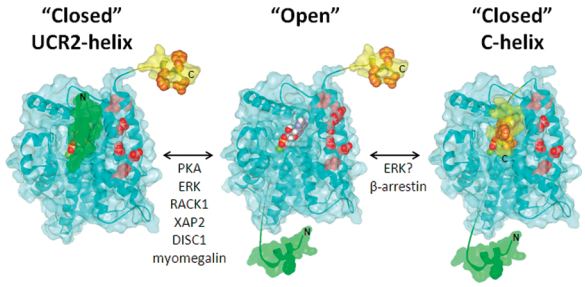


the active site, has been around for nearly half a century and is recognized as an important regulatory mechanism. For example, benzodiazepines such as Valium exert their powerful biological effects through allosteric binding to γ-aminobutyric acid receptors. However, when designing enzyme inhibitors, scientists typically go straight for the jugular and target the enzyme active site. While this strategy often yields potent inhibitors, such efficient inhibition can be a double-edged sword, leading to the emergence of drug-resistant variants or adverse side effects. Now, two studies (Burgin *et al.* (*Nat. Biotechnol.* 2010, *28*, 63−72) and Zhang *et al.* (*Nature* 2010, *463*, 501−506) describe the activity of allosteric inhibitors against two key therapeutic targets, demonstrating the striking benefits that can come from an allosteric approach.

In one study, Gurney and co-workers investigate allosteric inhibitors against the enzyme phosphodiesterase 4D (PDE4D). PDE4D hydrolyzes the second messenger cAMP and is an important therapeutic target for various brain disorders. While potent and therapeutically effective PDE4 inhibitors that bind the enzyme active site exist, the vomiting and diarrhea that they cause has precluded their development as drugs. In search of an alternative strategy, the authors examined the crystal structure of PDE4D with bound inhibitors, revealing that a regulatory domain in PDE4D functions by controlling access to the active site. The structures offered clues as to how the interaction of small molecules with this regulatory domain could modulate enzyme activity. Armed with this information, novel allosteric inhibitors were designed that only partially inhibited enzyme activity. The partial inhibitors were as effective as full inhibitors in altering cAMP signaling in cellular assays, and they provided cognitive benefit in rodent models of cognition. In contrast to full inhibitors, however, the tendency to vomit after exposure to the compounds was greatly diminished in animal models. The authors propose that by reducing the magnitude of PDE4D inhibition, signaling through cAMP is still effectively prevented but cellular cAMP levels remain sufficient to curb potential side effects.



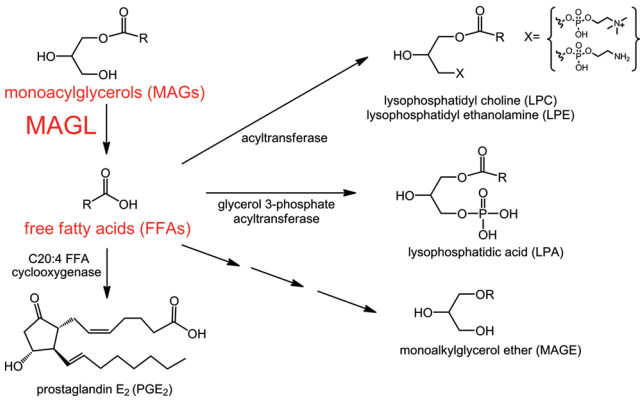
In another study, Gray and co-workers explore allosteric binding in Bcr-Abl, an oncogenic tyrosine kinase associated with chronic myelogenous leukemia (CML). Current drugs that target the Bcr-Abl ATP-binding site are effective against CML, but the emergence of drug-resistant forms of the protein has fueled the search for new inhibitors that function using alternative mechanisms. To this end, NMR spectroscopy and X-ray crystallography were used to explore the binding of an allosteric inhibitor of Bcr-Abl, GNF-2. The myristoyl binding pocket was identified as the binding site of GNF-2, and hydrogen-exchange mass spectrometry studies revealed that GNF-2 binding alters the conformational dynamics of the ATP-binding site, offering insight into its mechanism of action. When GNF-2 or the structurally related compound GNF-5 was used in combination with ATP-binding site inhibitors, Bcr-Abl kinase activity was inhibited *in vitro*, growth of cells containing a drug-resistant Bcr-Abl mutation was slowed, the emergence of drug resistance mutations was suppressed, and survival in a mouse-model of human CML was significantly enhanced. Together, the data suggest the combination of an ATP-binding site-directed and an allosteric inhibitor as a promising strategy for targeting oncogenic kinases.

The exciting findings reported in these studies point to allosteric binding as a powerful tool for manipulating protein activity. The ability of allosteric binders to tweak enzyme activity and work synergis-

tically with active site inhibitors opens the door to their use in other systems, where drug resistance or side effects present formidable challenges to drug discovery. **Eva J. Gordon, Ph.D.**

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.



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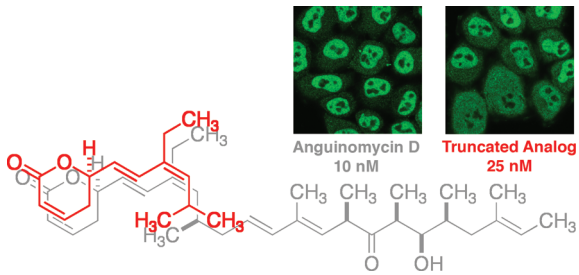
Comparing proteins found in cancer cell lines from a variety of aggressive and nonaggressive ovarian and breast tumors and melanomas, researchers found that the expression of monoacylglycerol lipase (MAGL) was consistently elevated in aggressive cancers. Inhibiting MAGL in aggressive cancer cells significantly 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. **Sarah A. Webb, Ph.D.**

Keeping Tumor Suppressors In

Many proteins that police tumor suppression must remain within the cell's nucleus to carry out their function. As cancer progresses, cells often acquire the ability to export these proteins into the cytoplasm. Over the past 25 years, researchers have widely investigated the leptomycin family of compounds because they can inhibit this process, but they proved too toxic for further investigation as drugs. However, in the original paper reporting their isolation, aguinomycins—with striking structural similarities to the leptomycin family—were reported to kill cancer cells but only stop growth in normal cells.



Reprinted with permission from Bonazzi, S., *et al.*, *J. Am. Chem. Soc.*, *132*, 1432−1442. Copyright 2010 American Chemical Society.

Bonazzi *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 1432−1442) have now synthesized the aguinomycins. These compounds inhibited the nuclear export of human protein Rio2 by CRM1 in HeLa cells at 10 nM concentrations. In addition, the researchers synthesized simplified analogues of the aguinomycins, which retained the lactone moiety with a shortened hydrophobic side chain or replaced the lactone with an α,β-unsaturated aldehyde but keeping the side chain, to evaluate which structural features confer these compounds' potency. Both analogues inhibited export at concentrations within an order of magnitude of those observed for the parent compounds. Because of the relative structural simplicity of the truncated lactone, this molecule represents a particularly attractive scaffold for a new drug.

In addition, the researchers used recently published X-ray crystal structure data to model the interaction between CRM1 and leptomycin B (LMB). Previous work had shown that LMB inhibits CRM1 through alkylation at cysteine 528, which is located in a groove on the surface of the protein which also recognizes nuclear export signals. Based on the potential hydrophobic interactions, LMB most likely binds with its entire hydrophobic tail nestled within this groove, and positively charged CRM1 residues orient the lactone ring for the cysteine's nucleophilic attack. Finally, the researchers synthesized a lactone analogue with a terpene-based hydrophobic

tail, which showed similar biological potency. Truncated analogues of anguinomyocins represent promising candidates for new potential cancer treatments that block nuclear transport. **Sarah A. Webb, Ph.D.**

Putting Together a Bacterial Organelle

Basic biology often teaches that a bacterium is a tiny bag of biochemical reactions, while the eukaryotic cell is a more elegantly organized life form with its extensive partitioning of reactions. Basic biology also teaches that nearly every rule that you find in a textbook is probably broken by some exception, and this generalization is no different.

Bacterial microcompartments are organelles measuring about 100 nm in diameter and they specialize in corralling certain biochemical pathways that warrant such control. One example, the ethanolamine utilization (Eut) pathway, is housed within a Eut microcompartment so that an intermediate in the process, acetaldehyde, cannot escape the cell due to its volatility. Losing that molecule would mean losing valuable carbon currency. Now, a structural study by Tanaka *et al.* (*Science* 2010, *327*, 81−84) with four *E. coli* Eut shell proteins shows how the cage is built.

The high-resolution crystal structures also revealed some interesting surprises. The EutM protein forms hexameric structures that could then fit together like a honeycomb, an architecture observed earlier in the microcompartment known as the carboxysome. The center of the hexamer is a pore that probably allows only very small molecules to pass. EutS displayed a hexameric structure as well, but with an unexpected kink in the donut shape. Previously unobserved in microcompartment proteins, this places six identical EutS proteins into three different structural environments. Similar to EutS, the EutL protein also formed hexamers but with an interesting twist. Two crystal forms generated structures with two different packing modes of the monomers. One form had a small pore size in the center, similar to EutM, but the other, more open form displayed an 8−11 Å pore that the authors postulate might aid in uptake of bulkier cofactor molecules into the Eut microcompartment. Finally, though the full-length EutK protein eluded the crystallographer's eye, the structure of the carboxy terminal domain of this protein revealed an interesting puzzle. The EutK terminus folds into a helix-turn-helix motif, a conserved nucleic acid binding motif found in all kingdoms of life.

So what is an ethanolamine utilization compartment doing binding to an RNA or DNA? The puzzle remains unsolved, but the parallels with viral capsid proteins that both enclose and bind to the viral genome may point this field in a new direction. This study displays the power of teasing apart a complex structural question by a sum-of-the-parts approach. **Jason G. Underwood, Ph.D.**