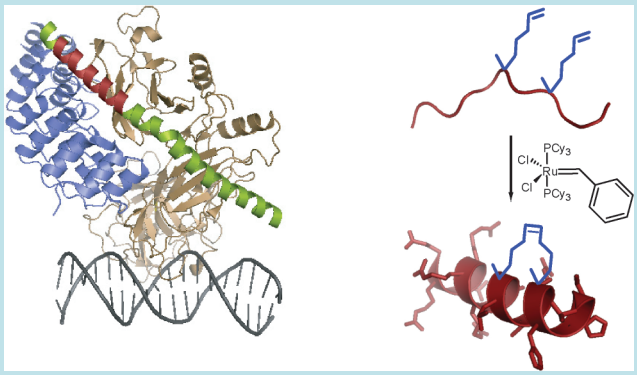
Spotlight

Stapled Helices Take Transcription Down a NOTCH



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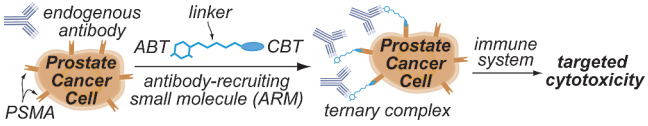
Traditional small molecule drugs tuck snugly into crevices and deep binding pockets. As a result, the broad, often shallow, terrain at most interfaces between proteins represents a tough target for drug development. Though protein–protein interactions are often involved in diseases such as cancer, most of these targets have been considered undruggable. One strategy for finding new candidates that specifically shut down a single pathway is to modify nature's schemes, by building a ligand whose structure matches the critical portion of a known binding partner. Moellering *et al.* (*Nature* 2009, *462*, 182–188) have now demonstrated how this approach blocks a proliferative pathway in cellular and mouse models of a human cancer.

The researchers used an α-helical peptide with a stabilizing chemical staple to target the transcription factor NOTCH, a transmembrane protein involved in signaling pathways that regulate cell fate. Mutations that activate NOTCH inappropriately are associated with many cancers including T-cell acute lymphoblastic leukemia (T-ALL). This intracellular portion of NOTCH can serve as a transcriptional activator, forming a complex with CSL, a DNA binding protein, and a coactivator, MAML1. To interfere with transcription, the researchers designed their inhibitory peptides, called SAHMs, based on a helix of MAML1. The peptides included two adjacent non-natural alkenyl amino acids, which are stapled together using ring-closing metathesis polymerization to stabilize the helical structure. A helix spanning Glu21 to Thr36 in MAML1 (SAHM1) proved most functionally active.

In a series of assays, Moellering *et al.* then demonstrated that SAHM1 competed with MAML1 to bind with the protein complex in vitro and in T-ALL cell lysates. SAHM1 also decreased the expression of NOTCH target genes in those cells. The researchers quantitatively analyzed the expression profiles, and the SAHM peptide were as effective in blocking transcription downstream of NOTCH as the effective, but less specific, γ-secretase inhibitors. Finally, SAHM1 treatment shut down the proliferation of T-ALL cells, which depend on NOTCH for growth, but did not affect the growth of cancer cells that were NOTCH-independent. In a mouse model of T-ALL, SAHM1 shut down NOTCH signaling and prevented disease progression. The results provide an innovative and general strategy for targeting a variety of diseases mediated by gene regulation. **Sarah A. Webb, Ph.D.**

Luring Antibodies to Prostate Cancer Cells

Over the past decade, drugs for a variety of cancers have been developed from monoclonal antibodies, which harness the body's immune system to attack cancer cells. Although these drugs have been effective in treating some cancers, they are expensive, can have severe side effects, and cannot be taken orally. Prostate cancer does not have an FDA-approved antibody treatment, and current treatment options often do not work against advanced disease.



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Murelli *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 17090–17092) applied a chemical approach that combines the best of both small-molecule and antibody-based chemotherapy strategies to prostate cancer. In another recent paper (*J. Am. Chem. Soc.* 2009, *131*, 16392–16394) researchers also in David Spiegel's laboratory at Yale University had demonstrated that this strategy can also enhance the immune response to HIV. In that work, they designed bifunctional small molecules, called ARM-Hs, that both inhibited interactions between proteins that mediate viral infection and formed an antibody-binding complex that enhanced the killing of infected cells.

Extending this idea to prostate cancer, the researchers designed molecules that both recruit antibodies and bind to prostate cancer cells, called ARM-Ps. For the antibody-binding end of the molecule,

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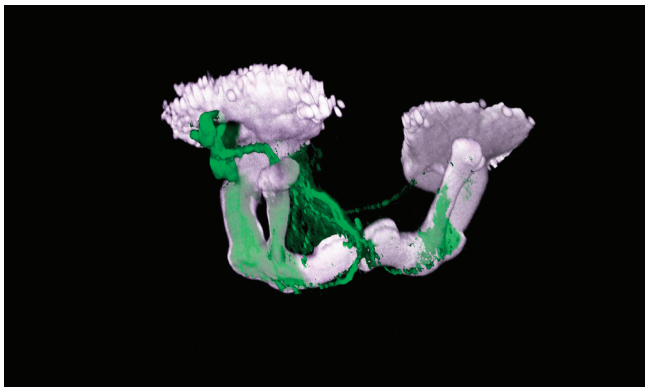
they chose a 2,4-dinitrophenyl (DNP) group because antibodies that respond to this antigen already circulate in the bloodstreams of a significant segment of the population. To bind to the cell, they chose a glutamate urea, which binds to prostate-specific membrane antigen (PSMA), a protein that is overexpressed on the surface of prostate cancer cells. Using computer modeling, Murelli *et al.* optimized the linker to include favorable interactions with the protein and to optimize number of oxyethylene groups in the linker to prevent steric clashes.

The researchers synthesized four molecules, with different linker lengths. ARM-P4, with four oxyethylene groups in the linker, showed the greatest binding to PSMA in a standard enzyme inhibition assay. However, in flow cytometry experiments, ARM-P8, with eight oxyethylene groups, maximized the formation of a ternary complex of cells expressing PSMA, linker molecule, and the antibody, anti-DNP. Finally, the researchers showed that ARM-P8 recruited antibodies that killed LNCaP prostate cancer cells but did not target cells that do not express PSMA. At high concentrations, the effectiveness of ARM-P8 drops off significantly, which suggests an autoinhibitory mechanism that could prevent overdoses. This creative chemical approach could provide a general template for safer cancer treatments. **Sarah A. Webb, Ph.D.**

Making Memories at the Laser Show

Teasing apart the wiring of a nervous system is a difficult task, but add in the less tangible aspects of neurobiology, such as learning and memory, and the challenges grow exponentially. Studies in model organisms like flies and worms have displayed how cues from the environment can relay through the animal's senses to trigger learning, but often the fine details remain mysterious.



A new study used an impressive approach to further dissect how memories are made in the fruit fly. Claridge-Chang *et al.* (*Cell* 2009, *139*, 405–415) first performed classical Pavlovian experiments that trained flies to associate a particular odor with a painful electric shock. Flies became conditioned to avoid the smell and could remember this association for several hours. Previous work showed that the neurotransmitter dopamine plays a role in aversive reinforcement, so the authors next honed in on dopaminergic neurons as putative memory relays. The authors turned to a modified fly strain carrying an ATP-responsive channel expressed only on the surface of dopaminergic neurons. A clever biochemical trick was then employed to regulate the function of these neurons with light. A caged-ATP molecule was microinjected and could be readily uncaged into active ATP at the flash of a laser. So, was pure dopamine signaling enough to teach a fly to avoid an odor? Amazingly, flies subjected to the odor stimulus while receiving a simultaneous laser pulse to trigger dopamine release learned to avoid that odor. In fact, these laser-instructed flies performed as well in the behavior tests as flies that had been trained by shock. Using different drivers to put the ATP-responsive channel into different sets of neurons, combined with neuroanatomy and a little help from the process of elimination, the study went on to map aversive reinforcement to the PPL1 cluster of 12 dopaminergic cells. This work demonstrates how the chemical biologist's toolkit can be used in a model organism to shine a bright light onto areas as complex as learning and memory. **Jason G. Underwood, Ph.D.**

Budding Yeast Harbor RNAi Machinery

The fields of RNA interference and high-throughput RNA sequencing have become powerful allies in recent years. Short RNAs are a perfect match for short read sequencing technologies, so increasingly more sequenced eukaryotic genomes are being accompanied by extensive collections of short RNA data. A notable exception has been the model budding yeast *Saccharomyces cerevisiae*, which does not harbor homologues of two key RNAi protein players, Dicer and Ago, in its genome. Other fungi display RNAi, and now, a new study indicates that several other budding yeast still possess a viable RNAi pathway and functional equivalents of these two key proteins.

Using size-fractionated RNA for sequencing, Drinnenberg *et al.* (*Science* published online Sept 10, 2009; DOI: 10.1126/science.1176945) showed that 22–23 nucleotide small interfering RNAs are present in other budding yeast species, including another *Saccharomyces* species and the human pathogen *Candida albicans*. A distantly related Dicer candidate was identified in these species and proved essential for generating small RNAs via genetic deletions combined with biochemical experiments. Interestingly, this new budding yeast Dicer lacks the PAZ domain, a portion of the protein that was previously proven important in plant or animal RNAi. The Ago proteins for the RNAi-capable yeasts were identified as well. The authors then undertook a large-scale loss-of-function assay by sequencing the entire mRNA population of an RNAi capable budding

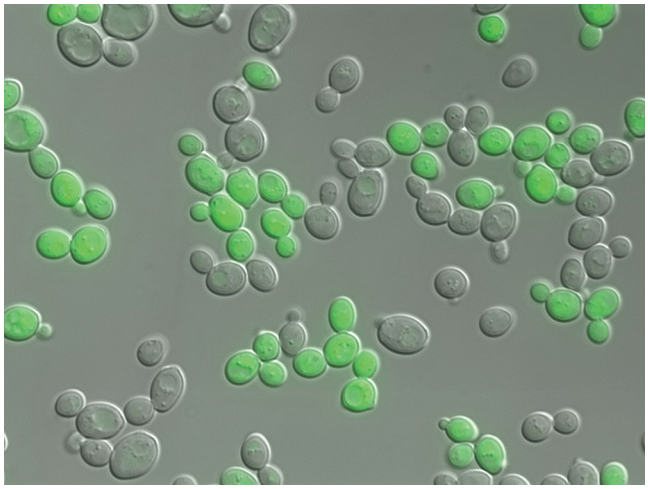
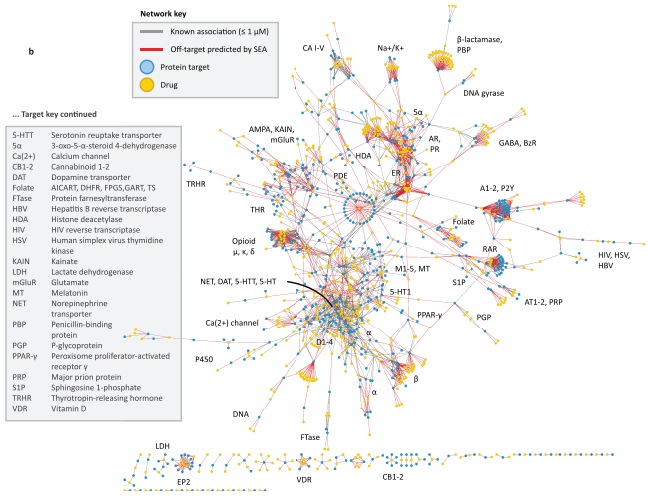


Image courtesy of authors.



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yeast, *S. castellii*, with or without a competent RNAi pathway. Only a few mRNAs changed, but conveniently, the loss of double-stranded RNA processing also helped pinpoint the transcription of precursors to the small RNAs. Over a thousand loci produce siRNAs, and the locations indicated that perhaps RNAi functions to silence transposable elements in the yeast genome, a function seen in other RNAi-capable organisms. Impressively, adding only the newly identified yeast Dicer and Ago proteins back into *S. cerevisiae* reconstituted RNAi in this organism. When this was performed, the engineered yeast showed lower accumulation of Ty transposon protein and mRNA, a silencing observation that was not possible in the RNAi yeast since most transposable elements were probably silenced long ago. Taken together, this study provides not only the tool of RNAi for the biologists studying yeast genes but also the tools of budding yeast to biologists studying RNAi. **Jason G. Underwood, Ph.D.**

Predicting Polypharmacology

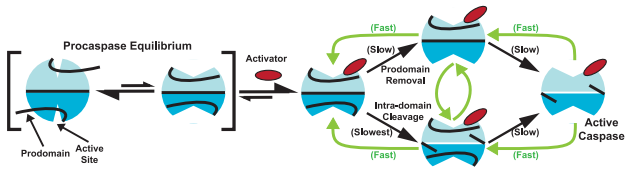
In an ideal world, a small molecule drug would interact only with its intended target, and side effects would cease to exist. In reality, most drugs bind to multiple targets, and this "polypharmacology" sometimes results in synergistic effects, while other times leading to outcomes so harmful as to abrogate use of the drug. Though a drug may be painstakingly designed to bind a specific target, once it enters the sea of biomolecules present in cells and tissues, accurately predicting just which targets it will find can be quite challenging. Keiser *et al.* (*Nature* 2009, *462*, 175–181) take on this challenge by developing a statistics-based chemoinformatics approach to predict all the targets of a given drug.

The approach, referred to as the similarity ensemble approach (SEA), classifies drug targets not by their structure or sequence, but by the similarity of the ligands that bind to them. Nearly 250 targets taken from the MDL Drug Data Report (MDDR) were classified by over 65,000 ligands, and the resulting ligand sets were analyzed against 3665 drugs, yielding nearly 1 million comparisons. Approximately 7000 pairs of drugs and ligand sets were identified as similar and were subsequently evaluated against known interactions and perused for unknown associations. For example, the compound brinzolamide is indicated only as an antiglaucoma agent in the MDDR; SEA analysis classified this compound with carbonic anhydrase inhibitors, and indeed, it is listed as a carbonic anhydrase inhibitor in the World of Molecular Bioactivity database. In addition, several unknown associations offered evidence for the origin of various drug activities, including the hallucinogenic properties of the metabolite DMT, the potential indication of the antihistamine Fabahistin as a drug for Alzheimer's disease, and the sexual dysfunction effects of the antidepressants Prozac and Paxil. **Eva J. Gordon, Ph.D.**

Suicide Activation

Proteases are typically synthesized as inactive precursors called proenzymes. Upon activation, these enzymes participate in numerous important biological events, including the process by which cells commit suicide, or apoptosis. Small molecule inhibitors of proteases are commonly used for investigating protease function; small molecules that directly activate rather than inhibit protease function could be powerful tools for exploring cellular processes. Wolan *et al.* (*Science* 2009, *326*, 853–858) now present the discovery of a small molecule activator of an enzyme involved in apoptosis, procaspase-3.

A screen of over 60,000 small molecules in search of those that could promote activation of procaspase-3 led to the identification of



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a trisubstituted coumarin derivative referred to as compound 1541. Compound 1541 was highly selective for procaspase-3 and the related enzyme procaspase-6, and various biochemical and kinetic experiments suggested that the compound stabilizes a more active conformation of the inactive enzyme while also rendering it more susceptible to proteolysis. A wide range of cancer cell lines underwent apoptosis upon exposure to 1541, and subsequent genetic experiments offered compelling evidence that the compound did indeed act through activation of procaspase-3. Moreover, initial studies suggested that cancer cells were more susceptible to apoptosis in response to compound 1541 than normal cells, hinting at a promising future for such compounds as potential chemotherapeutic agents. Given the pivotal roles of proteases in other biological processes, such as blood clotting and cell differentiation, the search for small molecule protease activators presents an innovative approach to manipulate protease function and is an exciting new strategy for drug discovery efforts. **Eva J. Gordon, Ph.D.**