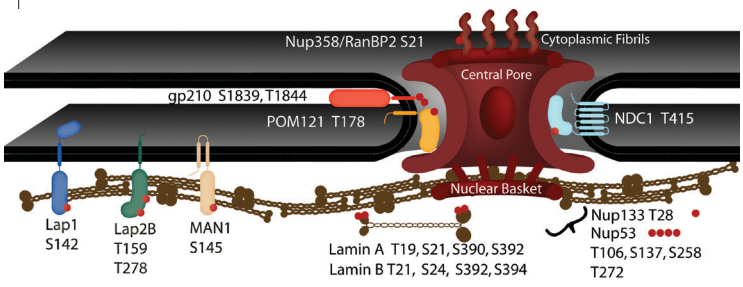
Spotlight

Circumventing Cysteine

Given the crucial role that kinases play in biological processes, it sure would be nice to have a comprehensive map of all phosphorylation sites and kinase–substrate pairs. One significant challenge in achieving this goal is identifying the many phosphorylation events that occur at low stoichiometry and on low-abundance proteins. Now, Blethrow *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2008, *105*, 1442–1447) describe an innovative method for the identification of substrates for Cdk1-cyclinB, a kinase that phosphorylates a large, diverse set of proteins, many of which are present in small quantities.

To differentiate Cdk1-cyclinB among the plethora of kinases in complex biological mixtures, the authors used an engineered Cdk1-cyclinB mutant designed to bind an ATP analogue. To identify Cdk1-cyclinB substrates, they designed the ATP analogue to also transfer a thiophosphate, rather than a phosphate, to the substrate. Enzymatic digestion yields thiophosphate-containing peptides that can be captured using a thiol-reactive, solid-phase electrophile. One catch is that cysteine-containing peptides can also participate in this reaction, forming thioether attachments to the solid phase. The authors cleverly exploit the different reactivities of these alkylation products upon oxidation, which causes hydrolysis of the thiophosphopeptide substrates and subsequent liberation into solution, while the thioether linkages remain stable and attached to the solid support. To demonstrate the utility of this approach, they identified 72 proline-directed Cdk1-cyclinB phosphorylation sites in HeLa cell extracts, including a mixture of previously known substrates and new candidate substrates. Notably, many of the putative substrates do not possess an optimal Cdk consensus sequence and thus would not be identified as Cdk1 phosphorylation sites based on sequence analysis alone. The method was also used to search for Cdk substrates in the nuclear envelope, where again known and unknown substrates were identified. **Eva J. Gordon, Ph.D.**



Blethrow, J. D., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 1442–1447. Copyright 2008 National Academy of Sciences, U.S.A.

A Good Offensive Strategy

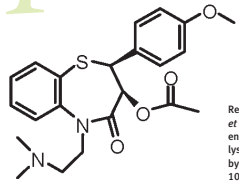
It is often said that the best defense is a good offense, and the offensive tactic of vaccination remains one of the best methods to combat viral diseases. The most effective vaccination strategy involves immunization with a live, attenuated virus, but no systematic approach for obtaining attenuated viral strains exists. Moreover, the high mutation rates of RNA viruses can result in reversion of attenuated vaccine strains to the wild-type pathogenic phenotype, putting unvaccinated communities at risk. By crippling the ability of the virus to mutate so rapidly, Vignuzzi *et al.* (*Nat. Med.*, 2008, *14*, 154–161) report a novel approach for creating attenuated viral vaccines.

Previous work in poliovirus revealed that when a key residue in the viral RNA-dependent RNA polymerase was mutated, the resulting strain possessed an attenuated phenotype because of increased replication fidelity and decreased genetic diversity. The authors reasoned that viruses with increased replication fidelity could represent a general class of attenuated vaccines and thus engineered several poliovirus strains with increased RNA polymerase fidelity. Direct

Published online March 21, 2008 • 10.1021/cb800042d CCC: $40.75

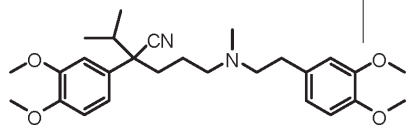
acs chemical biology

Teaching an Old Drug New Tricks



Reprinted from *PLoS Biol.*, Mu, T. W., *et al.*, Partial restoration of mutant enzyme homeostasis in three distinct lysosomal storage disease cell lines by altering calcium homeostasis, DOI: 10.1371/journal.pbio.0060026.

1 (Diltiazem)



Verapamil

Gaucher disease is one of >40 lysosomal storage diseases (LSDs) for which no ideal drug treatment options exist. At the roots of this disease are mutations that result in a lack of the active enzyme, glucocerebrosidase (GC), in lysosomes. A key feature of many of the disease-related enzyme variants is that they are improperly folded and trafficked upon translation. Because properly folded variants of these mutant enzymes retain activity, this suggests that enhancing protein folding and trafficking is a way to treat this disease and other LSDs. Building on growing evidence for the role of calcium ion signaling in enhancing protein folding and trafficking, researchers in Jeffrey Kelly's lab examine whether perturbing calcium homeostasis ameliorates enzyme function in Gaucher disease and two other LSDs, with quite striking results (*PLoS Biol.*, published on Feb 5, 2008; DOI: 10.1371/journal.pbio.0060026).

The authors discovered that two L-type Ca^{2+} channel blockers, diltiazem and verapamil, currently used to treat high blood pressure, enhanced GC activity in Gaucher patient-derived cell lines. In these cell lines, 10 μM diltiazem resulted in increase of GC activity by ~2-fold. The authors showed that the increase in activity was due to Ca^{2+} channel blocker activity and not solely structure. In addition, the authors demonstrated that this increase in activity in a dose-dependent manner is a result of proper folding and trafficking and not due to increased transcription of the mRNA or binding-induced stabilization of the enzyme. The last point is an important one, because it immediately suggested that diltiazem might be used in treating other diseases that result from enzyme misfolding and mistrafficking. This point is not lost on the authors—they demonstrated the potential for using this drug in two other LSDs.

Too often, small molecules fail to exhibit the properties required to be effective and practical drugs. Perhaps the most profound result of this study is that it suggests the possibility of using two drugs, already in widespread global use, for new purposes. **Anirban Mahapatra, Ph.D.**

sequencing of the genomes of these strains revealed that the mutation frequency of the variants was significantly reduced relative to the wild-type strain, resulting in a restricted viral population diversity. It was next demonstrated that while maintaining similar replication kinetics, the strains with increased replication fidelity were compromised in their ability to mutate, decreasing their ability to evolve toward a more pathogenic genotype. Finally, the attenuated viruses were tested for virulence and their ability to produce an immune response in mice. It was shown that all high-fidelity viruses were strongly attenuated. In addition, they exhibited a marked reduction in viral shedding, indicating a reduced risk of introduction of the virus into the environment. Most encouraging, however, was the demonstration that the high-fidelity variants induced poliovirus-specific neutralizing antibodies and protected vaccinated mice from lethal injection of wild-type poliovirus. This method provides an exciting new offensive strategy for the rational design of vaccines and could be applied to a wide variety of viruses. **Eva J. Gordon, Ph.D.**

Spotlight

RNAi Libraries Take on Cancer

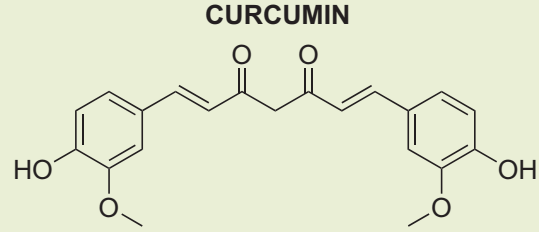
Large-scale genetic screens used to be a trick reserved for model organism tinkering. Biologists poked at the genetic makeup of yeast, worms, or flies with chemical mutagens or with DNA insertions landing somewhere in the genome. After a desired mutant was found, the hunt for what gene caused that phenotype was an additional chore. Researchers studying human cancers have adopted genetic and chemical screening approaches as well, but often guided by candidate genes or families of genes in a common pathway. But what if the key to a cancer's growth is a previously uncharacterized gene that has mutated or lost its proper regulatory switches? Now, a new unbiased screening approach combines three of the most powerful tools available to molecular biologists, RNA interference (RNAi), retroviral infection, and microarrays to assay cancer cells for essential genes.

Two new studies (Silva *et al. Science* 2008, *319*, 617–620, and Schlabach *et al. Science* 2008, *319*, 620–624) use a large short-hairpin RNA (shRNA) library in which each shRNA targets a predicted protein-coding gene in the human genome for degradation. The delivery method, a modified retroviral construct, ensures that one shRNA cassette is stably integrated into the genome of a single cancer cell. Each cassette is also tagged with a special "barcode" sequence to allow later identification by microarray hybridization. The study by Silva and coworkers applied the shRNA library to several breast cancer cell lines, and the Schlabach study used a breast cancer line, normal breast epithelial cells, and two colon cancer lines to allow comparative analyses. Integration of the shRNA knockdown cassette was assayed by PCR amplification on genomic DNA after initial infection utilizing primers that flank the shRNA and barcode. After a few days, the same amplification was performed and compared with the initial sample by microarray. Any shRNA sequence that "dropped out" over time probably killed or hindered cell growth, and the targeted gene could be identified. In a comforting confirmation of the method, many known essential genes such as cell-cycle regulators were identified, but with >100 gene hits in many of the cell lines, there are plenty of new candidate genes for further exploration. Some were chosen for validation experiments using new shRNA sequences to control for any off-target effect that may have caused a false positive. Each cell type showed a different shRNA susceptibility signature with some overlap, as expected. Most interesting from a therapeutic standpoint were the genes required for proliferation of the breast cancer cells but not normal breast endothelial cells. These new candidate genes will surely be the subject of future investigations, but with this new shRNA library available, this screen is only the tip of the iceberg.

While this screen just asked for cells to drop out of the pool, many clever ideas could make use of this same retroviral shRNA library but coupled with a different phenotypic screen. Also, these studies only touched upon a few of the cancer cell lines that currently reside in common cell banks. Developing signatures for other cell lines and comparing similar tumor types may help find the underlying functional defect in various cancers. It's been over 30 years since Nixon declared war on cancer, but clearly, with this type of large-scale unbiased screen and specific "Cancer Genome Projects", new smart bombs are being escorted to the battlefield. **Jason G. Underwood, Ph.D.**

Hot and Spicy Cancer Cure?

Curcumin is the major active constituent of turmeric, the spice most readily known for the flavor it provides in curry dishes. Turmeric has been used in Asian medicine for generations to treat many diseases, including cancer. More recently, Western medicine studies have provided evidence for curcumin's anticancer properties by demonstrating that it interacts with the cancer targets DNA topoisomerase I (topo I) and II (topo II). Topo I and II play crucial roles in replication, transcription, recombination, and chromatin remodeling, and several topo poisons, such as etoposide and camptothecin derivatives, are



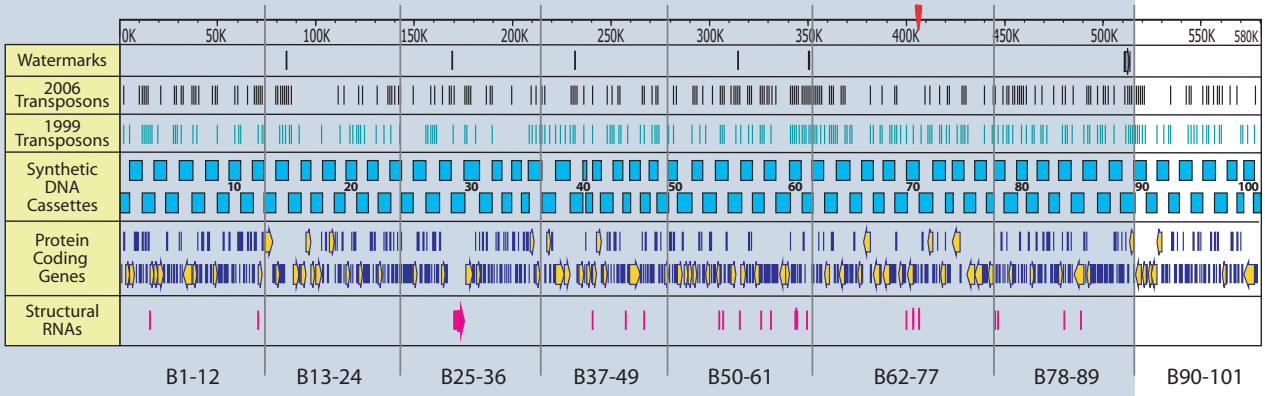
used as chemotherapeutic agents. López-Lázaro *et al.* (*J. Nat. Prod.* 2007, *70*, 1884–1888) now investigate the mechanism by which curcumin kills cells, providing insight into how this compound could be used in the treatment or prevention of cancer.

The authors use an immunofluorescence assay (the TARDIS assay) to investigate whether curcumin, like other topo poisons, induces the formation of topo I– or topo II–DNA complexes in a leukemia cell line. Indeed, curcumin induced DNA complexes with both topo I and topo II in a time- and concentration-dependent manner, an exciting finding given that other topo poisons typically target either topo I or topo II, but not both. Moreover, the curcumin-induced topo I– and topo II–DNA complexes remained stable after compound removal, in contrast to those induced by other topo poisons. These studies provide compelling data that curcumin may have potential as an anticancer agent. Thus, while it may be tempting to eat a bunch of curry dishes in a simultaneous battle against hunger and cancer, it should be noted that the amount of curcumin needed for preventative or therapeutic effects likely far exceeds that which can be consumed in food. Eva J. Gordon, Ph.D.

Chemical Genomes

Efforts to determine the essential genetic functions for life would be greatly facilitated if we could assemble entire genomes from scratch. Although completely synthetic viral genomes have been reported, assembly of bacterial genomes presents a significant challenge because of their substantially larger size. Gibson *et al.* (*Science*, 2008, *319*, 1215–1220) now report the complete chemical synthesis of the nearly 600,000 base pair genome of the bacterium *Mycoplasma genitalium*.

The strategy for genome construction began by breaking the DNA sequence down into 101 cassettes of 5–7 kb each. To simplify the future deletion or manipulation of the genes, each cassette contained one or more complete genes. Short "watermark" sequences, which are sequences used to encode information into DNA, were inserted into several cassettes to enable the differentiation of the synthetic genome from the native genome. In addition, an aminoglycoside resistance gene was added within the MG408 gene, which both eliminated the pathogenicity associated with the genome and allowed for selection during cloning. Once these pieces were in place, genome assembly proceeded in four stages. In the first three stages, the cassettes were linked



From Gibson, D. G., *et al.*, *Science*, Jan 23, 2008, DOI: 10.1126/science.1151721. Reprinted with permission from AAAS.

together by *in vitro* recombination and cloning in *Escherichia coli*. First, sets of four neighboring cassettes were assembled, resulting in 25 assemblies. This was followed by assembly of sets of three to yield eight assemblies. Finally, sets of two were joined together to form four assemblies, each of which composed approximately one-fourth of the genome. Because of difficulties in assembling the rest of the genome in *E. coli*, the final assembly was carried out in yeast by TAR cloning, in which the one-fourth genomes and vector DNAs are co-transformed into yeast spheroplasts and then joined by homologous recombination. The final 600-kb genome was purified from yeast and sequenced for verification. This genome represents the largest chemically synthesized molecule of defined structure reported to date. **Eva J. Gordon, Ph.D.**