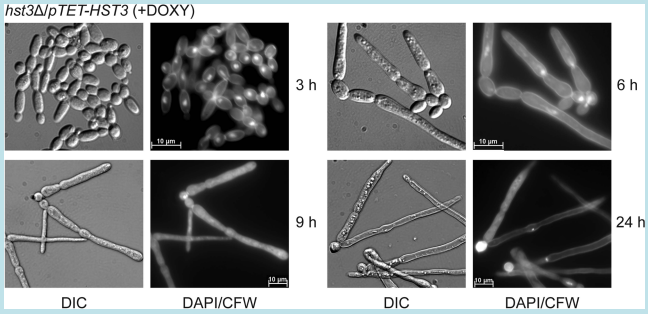
Spotlight

Hitting Up Histone Acetylation for Antifungals



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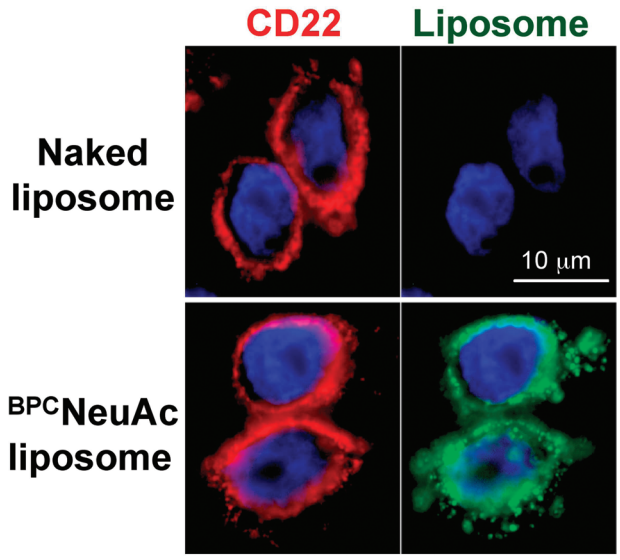
Not to be outdone by bacteria and viruses, fungi have also devised methods to resist their own demise by antifungal drugs. Indeed, increasing numbers of strains of the fungal pathogen *Candida albicans*, the fourth most common cause of hospital-related bloodstream infections, develop resistance to drugs that target the biosynthesis of ergosterol or of the fungal cell wall. New fungal drug targets are needed to discover novel drugs capable of circumventing these resistance pathways. Now, Wurtele *et al.* (*Nat. Med.* 2010, *16*, 774–780) identify two enzymes involved in histone acetylation as intriguing antifungal drug targets.

Immunoblotting and mass spectrometry were initially used to confirm that the post-translational modification H3K56ac, in which lysine 56 of histone H3 is acetylated, is present in *C. albicans*. Comparison with *Saccharomyces cerevisiae* and genetic experiments next indicated that *RTT109* and *HST3* are the genes that encode the acetyltransferase and the deacetylase, respectively, responsible for the dynamic acetylation of H3K56. Studies of various *RTT109* and *HST3* deletion mutants revealed that perturbation of H3K56ac leads to increased sensitivity to genotoxic and antifungal drugs. Further studies suggested that repression of *HST3* leads to a state of hyperacetylation that has disastrous consequences such as histone degradation, DNA fragmentation, and ultimately cell death. The deacetylase encoded by *HST3* is a member of the sirtuin family of histone deacetylases, which are inhibited by the vitamin nicotinamide. Indeed, exposure of *C. albicans* cells to nicotinamide resulted in toxicity similar to that seen with *HST3* repression, implicating this class of molecules as potential antifungal agents. To this point, mice in which either *HST3* or *RTT109* were repressed, or that were treated with nicotinamide, were protected from *C. albicans* infection. These results point to histone acetylation as a promising new target for combating fungal infections. **Eva J. Gordon, Ph.D.**

A Sugar-Coated Anticancer Strategy

Blood cancers account for nearly 10% of cancer diagnoses and cancer-related deaths. Though much progress has been made in combating leukemias and lymphomas, which originate in disease-fighting white blood cells such as B-cells, better treatments and cures are still desperately needed. Exploiting a carbohydrate-binding interaction on the surface of malignant B-cells, Chen *et al.* (*Blood* 2010, *115*, 4778–4786) report a "sugary" twist on a nanoparticle-based therapeutic strategy for attacking B-cell leukemias and lymphomas.

B-cells display the carbohydrate-binding receptor CD22 on their surface. CD22 binds to α2–6-linked sialic acid residues, and this interaction accelerates the constitutive endocytosis of the receptor. Building off clinically used anticancer liposomal formulations that encapsulate the chemotherapeutic agent doxorubicin, similar liposomes were coated with sialic acid containing glycans specific for CD22 on their surface. It was speculated that interaction of the liposome with CD22 would be followed by endocytosis and conse-



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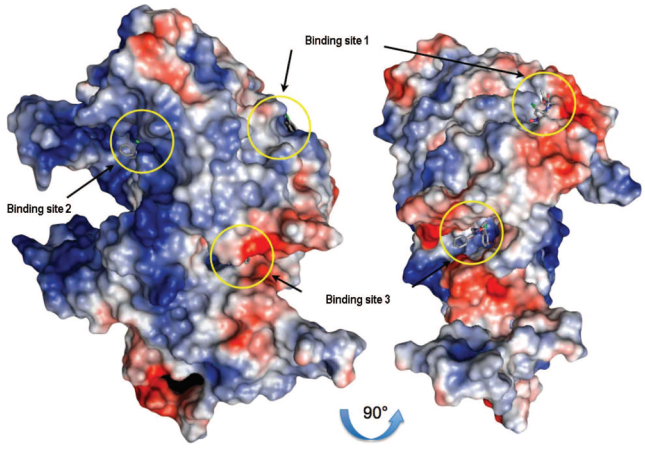
quent delivery of the chemotherapeutic cargo directly inside the malignant cell. Indeed, it was demonstrated that the sugar-coated liposomes bound and killed cells from a lymphoma cell line *in vitro* and effectively targeted lymphoma cells in mouse whole blood samples and in live mice. Moreover, treatment with the liposomes considerably prolonged life in mouse models of lymphoma. Finally, the liposomes also effectively targeted and killed cancerous B cells from the blood of patients suffering from hairy cell leukemia, chronic lymphocytic leukemia, and splenic marginal zone lymphoma. These promising results validate this "sugar-coated" strategy for targeting B-cells, which shows unique promise for the development of improved therapeutic agents for B-cell lymphomas and leukemias. **Eva J. Gordon, Ph.D.**

Escaping Viral Resistance

A disconcerting number of bacteria and viruses have devised methods to outsmart drugs targeted against them. These pathogens often acquire resistance by adopting mutations in a drug target that diminish interaction with the drug but leave their pathogenicity intact. This trend has led to a growing need for new bacterial and viral drug targets as well as new small molecule inhibitors of such targets. Kao *et al.* (*Nat. Biotechnol.* 2010, *28*, 600–605) address this need by identifying nucleoprotein, the most abundantly expressed protein during the infection process, as a new drug target for influenza A and discovering an effective new small molecule nucleoprotein inhibitor.



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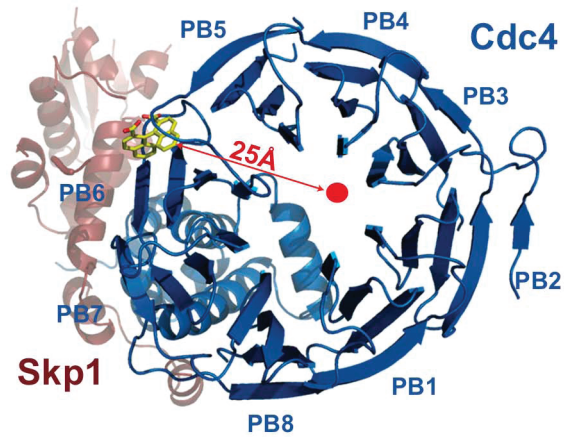
In a forward genetics approach, canine kidney cells infected with influenza A were screened with a library of over 50,000 structurally diverse compounds for molecules capable of preventing infection. Using fluorescence microscopy, of 39 inhibitors identified, several were found to prevent the accumulation of nucleoprotein in the nucleus. One compound, nucleozin, was studied further to gain insight into the mechanism of action of these compounds. Molecular modeling studies based on the crystal structure of nucleoprotein pointed to three potential binding sites for nucleozin. Creation of mutant "escape" viruses resistant to nucleozin activity led to the identification of a single tyrosine to histidine mutation that conferred resistance, which implicated a small groove behind the body of nucleoprotein as the nucleozin binding site. Nucleozin binding may cause a conformational change that promotes aggregation of the protein and consequent exclusion from the nucleus. Notably, treatment with nucleozin protected mice infected with the highly virulent H5N1 influenza A strain. The exquisite details of the interaction between nuclezoin and nucleoprotein illuminated in this study will facilitate the design of drug candidates for nucleoprotein variants in other viruses as well. **Eva J. Gordon, Ph.D.**

Propelling the Way to Ubiquitination Inhibitors

Ubiquitination lies at the heart of the intracellular protein degradation machinery, which is critical for maintaining proper protein homeostasis, ridding the cell of improperly folded proteins, and enabling changes of cell state in the cell cycle and development. The hundreds of proteins that become ubiquitinated and the diverse functions in which they participate make this system attractive for drug discovery efforts. Specificity in the ubiquitination process is dictated by the ubiquitin ligases that attach ubiquitin to appropriate substrate proteins. Orlicky *et al.* (*Nat. Biotechnol.* 2010, *28*, 733–737) now present the discovery of SCF-I2, a small molecule inhibitor of the yeast ubiquitin ligase Cdc4, and solve the crystal structure of SCF-I2 bound to Cdc4.



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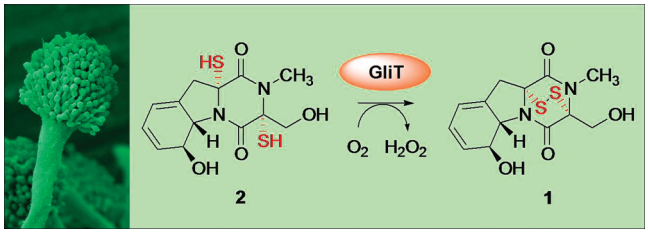
SCF-I2 was identified using a fluorescence polarization assay, in which 50,000 compounds were screened for their ability to displace a fluorescent peptide bound to yeast Cdc4. A derivative of BINOL,

a commonly used ligand in asymmetric synthesis, SCF-I2 was found to be a selective, allosteric Cdc4 inhibitor. SCF-I2 bound to a site located 25 Å from the substrate binding site, in which phosphorylated substrate motifs (referred to as the CPD) interact. Cdc4 contains a WD40 repeat domain that forms a circular "β-propeller" structure; the crystal structure reveals that SCF-I2 inserts between blades 5 and 6 of the propeller. This binding event induces large conformational changes that ultimately disrupt recognition of the CPD motif. Mutational and structure–activity studies highlight the importance of the naphthalene rings of SCF-I2 for hydrophobic interactions, and the carboxylate ions for electrostatic interactions. The specificity of SCF-I2 is illustrated by the fact that it is only a weak inhibitor of the human analogue of Cdc4, due to the loss of key nonconserved residues needed to engage SCF-I2. The structural and mechanistic insights into the Cdc4-SCF-I2 interaction should facilitate the design of other inhibitors in related systems and provide a point of departure for drugs that target substrate recognition in the ubiquitination system. **Eva J. Gordon, Ph.D.**

Potency and Self-Resistance in a Single Package

Pathogenic fungi acquire their potency from toxins with unique chemical features. One fungal infection, invasive aspergillosis, is particularly dangerous for patients with compromised immune systems. The fungus involved, *Aspergillus fumigatus,* produces gliotoxin, a poison that includes unusual transannular disulfide bonds. Now Scharf *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 10136–10141)



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have explained both how these bonds are formed in the fungus and how that biochemical mechanism prevents the fungus from succumbing to its own toxin.

Gliotoxin damages host cells either through a reactive oxygen species or by modifying critical enzymes within the cytosol. Analysis of the gliotoxin biosynthesis gene cluster pointed to *gliT*, a gene whose corresponding amino acid sequence was similar to an oxidoreductase. A mutant lacking this gene cannot produce gliotoxin. *In vitro*, the enzyme did not act as a reductase, either with gliotoxin or with other substrates, but as an oxidase, converting a reduced dithiol precursor compound to gliotoxin. The enzyme uses an FAD cofactor, but unlike similar oxidoreductases, the enzyme does not bind to NADPH. Instead, it uses molecular oxygen as the terminal electron acceptor.

Further studies with the mutant lacking *gliT* offered an explanation for the resistance of the fungus to its own toxin. The researchers used this mutant to look for accumulation of the dithiol, as confirmation that the enzyme built the disulfide bridge. Because the reduced gliotoxin can react with thiol side chains within proteins, the molecule was only detected when additional gliotoxin was added to the culture. However, the researchers also noticed that the *gliT* knockout fungus grew much more slowly than wild type when gliotoxin was present. In an inhibition assay, the researchers confirmed that the GliT enzyme was critical for the fungal resistance to the toxin.

GliT is the first enzyme that is been shown to form this type of disulfide, but related compounds containing this disulfide bridge, epipolythiodioxopiperazines, are produced by other fungi. Because of its role in both virulence and self-resistance, this enzyme, or other enzymes with similar activities, could serve as a target for new antifungal therapies. **Sarah A. Webb, Ph.D.**