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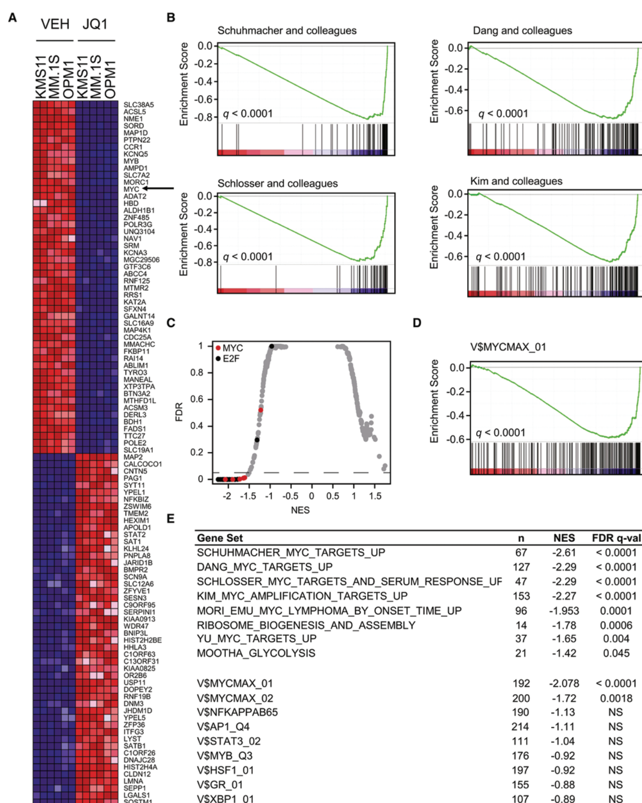
All BETs are Off

c-Myc is a transcription factor that regulates the expression of an impressive 15% of all genes, orchestrating key pathways in cell growth and survival. Overexpression of c-Myc is notorious for its role in numerous cancers, but efforts to target the oncogene for therapeutic benefit have been thwarted by challenges associated with disrupting its interaction with its partner transcription factor. Interestingly, in c-Myc's purview is the regulation of histone lysine acetylation, a process that itself is intricately and profoundly associated with global gene transcription. Exploration of this relationship led Delmore *et al.* (*Cell* 2011, *146*, 904−917) to discover a promising new strategy for targeting c-Myc.



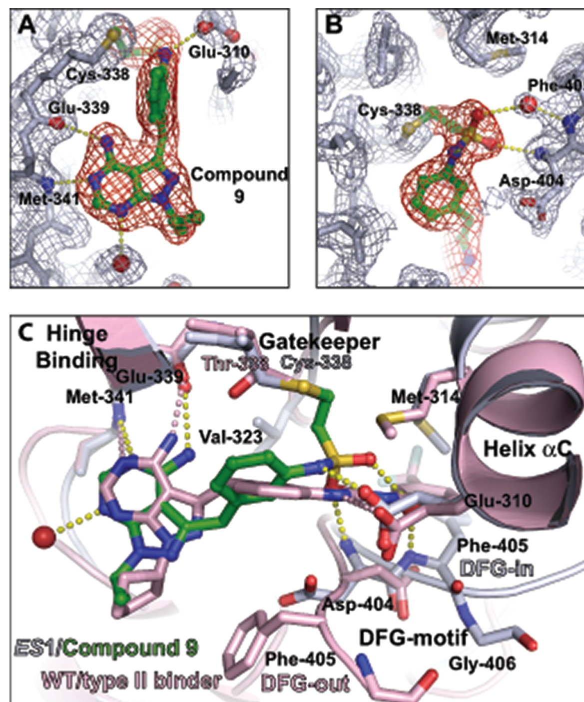
Gene Set	n	NES	FDR q-val
SCHUHMACHER_MYC_TARGETS_UP	67	-2.61	< 0.0001
DANG_MYC_TARGETS_UP	127	-2.29	< 0.0001
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UF	47	-2.29	< 0.0001
KIM_MYC_AMPLIFICATION_TARGETS_UP	153	-2.27	< 0.0001
MORI_EMU_MYC_LYMPHOMA_BY_ONSET_TIME_UP	96	-1.953	0.0001
RIBOSOME_BIOGENESIS_AND_ASSEMBLY	14	-1.78	0.0006
YU_MYC_TARGETS_UP	37	-1.65	0.004
MOOTHA_GLYCOLYSIS	21	-1.42	0.045
V$MYCMAX_01	192	-2.078	< 0.0001
V$MYCMAX_02	200	-1.72	0.0018
V$NFKAPPAB65	190	-1.13	NS
V$AP1_Q4	214	-1.11	NS
V$STAT3_02	111	-1.04	NS
V$MYB_Q3	176	-0.92	NS
V$HSF1_01	197	-0.92	NS
V$GR_01	155	-0.88	NS
V$XBP1_01	107	-0.89	NS

Reprinted from *Cell, 144*, Delmore, J. E, *et al.*, BET Bromodomain Inhibition as a Therapeutic Strategy to Target c-Myc, 904−917. Copyright 2011, with permission from Elsevier.

Histone acetylation triggers the assembly of large transcriptional complexes, composed in part of human bromodomain and extra-terminal (BET) proteins that bind to the acetylated chromatin. Recent identification of a small-molecule triazolodiazepine called JQ1, which prevents BET bromodomains from binding to chromatin, and cellular and animal models of multiple myeloma, a type of blood cancer in which c-Myc dysfunction has been implicated, provided compelling chemical and biological tools to explore the link between BET bromodomains and c-Myc activity. First, gene expression analysis demonstrated the involvement of the BET bromodomain BRD4 in multiple myeloma. Next, global transcriptional profiling experiments showed that BET bromodomain inhibition by JQ1 results not only in the selective repression of c-Myc-dependent transcriptional networks but surprisingly in the direct inhibition of *MYC* gene expression as well. RNA inhibition and chromatin immunoprecipitation experiments further suggested that BRD4 is a coactivator of *MYC* gene expression. In addition, JQ1 treatment of multiple myeloma cell lines resulted in decreased cell growth and viability concurrent with suppression of c-Myc expression. Finally, in several mouse models of multiple myeloma, JQ1 treatment led to various beneficial responses including decreased tumor burden and prolonged survival. This study establishes important proof of concept for a new approach to attacking a notoriously elusive cancer target. **Eva J. Gordon, Ph.D.**

Covalent Complemetarity

Selective inhibition of enzyme activity is critical in studies of cellular processes, as promiscuous interactions of inhibitors with biomolecules other than their intended target can complicate the interpretation of experimental results. Many of the over 500 protein kinases present in the human proteome, which use ATP to phosphorylate proteins targets, are notoriously difficult to inhibit selectively due to the conserved nature of the ATP-binding site of the enzyme, where most inhibitors bind. Chemical genetic approaches for creating selective kinase inhibitors by enlarging the kinases's ATP binding site pocket have achieved great success in delineating the roles of specific kinases but in some cases such manipulation results in loss of kinase activity. Garske *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 15046−15052) now report a related strategy that relies on the introduction of a cysteine in the kinase active site and the design of complementary electrophilic inhibitors.



Garske, A. L., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *108*, 15046-15052. Copyright 2011 National Academy of Sciences, U.S.A.

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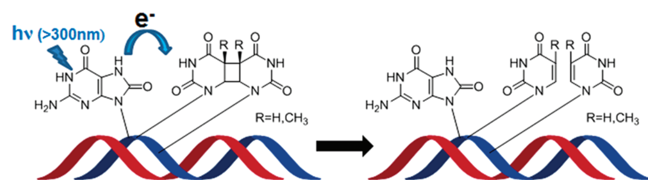
Cysteine was selected for placement into the ATP binding site of due to its unique chemical reactivity, the natural lack of cysteines in most kinase ATP binding sites, and its relative hydrophobicity, deemed important for conformational stabilization of the enzyme. Cysteine was introduced into the active site of the protein kinase Src, and pyrazolopyrimidine and 4-anilinoquinazoline scaffolds were chosen for the design of compatible electrophilic inhibitors of the mutant enzyme. Vinylsulfones, acrylamides, chloroacetamides, and fluoromethylketones were explored as electrophiles, leading to the discovery of several potent, irreversible inhibitors. An X-ray structure of the complex with a vinylsulfone-containing inhibitor elucidated the details of the interaction, and confirmed covalent attachment of the inhibitor. Screening of a panel of over 300 kinases with five of the inhibitors showed limited inhibition of other kinases. Experiments in cells expressing the mutant kinase demonstrated selective inhibition in a cellular context. Notably, inhibitor potency could be improved with strategic additional mutations in the active site designed to optimize the environment for nucleophilic chemistry. **Eva J. Gordon, Ph.D.**

Shining a Light on Repair

The hypothesis of a primordial RNA World stems from the unique property of RNA to act as both a catalyst and a carrier of genetic information. Today, critical cellular functions are known to utilize ribozyme catalysis and *in vitro* evolution experiments have uncovered dozens of reactions that could have been catalyzed by ribozymes on ancient Earth. In addition, many protein enzymes depend upon nucleotide cofactors to facilitate redox reactions. In a recent study, Nguyen and Burrows (*J. Am. Chem Soc.* 2011, *133*, 14586−14589) took the protein out of the equation to study a redox reaction with important implications to primordial nucleic acid replication.



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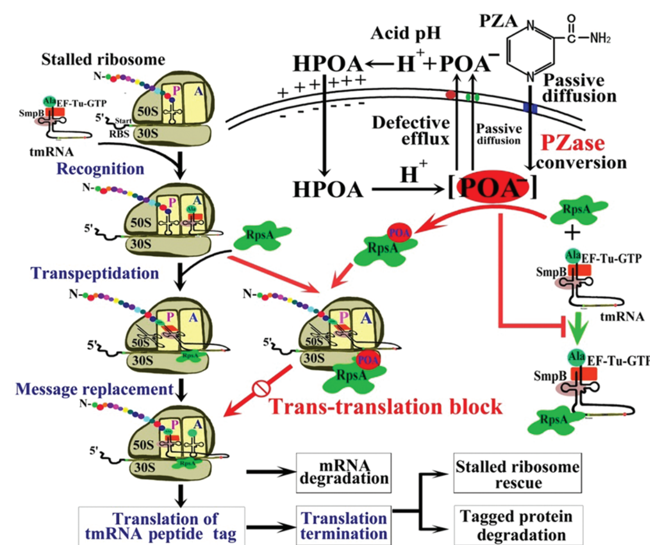
The reaction was to repair a thymine dimer in a DNA duplex, but without the benefit of the usual enzyme, photolyase, or its electron-donating cofactor, FADH. In lieu of the flavin, the thymine dimer was placed near an 8-oxo-7,8-hydroguanine (OG) in the DNA duplex. This nucleobase, thought to be present on early Earth, displays a much lower redox potential, *i.e.*, is more easily oxidized, than the canonical A, G, T, and C found in natural DNA. The OG base is also unique in that it shows significant absorbance at wavelengths of light above 300 nm, whereas natural nucleotides do not. Interestingly, such irradiation of a DNA duplex containing adjacent OG and thymine dimer resulted in DNA repair of the dimer site to obtain two thymidine bases, just as occurs with photolyase repair. The researchers went on to show that OG could function in an intra- or interstrand fashion to repair a dimer and that multiple turnover catalysis was possible, indicating that the OG redox potential is restored during dimer repair. Dissecting the light-induced reaction further indicated that repair was also possible on RNA U−U dimers and works most efficiently when the OG is paired with an A rather than a C and placed 5′ of the dimer site. This study indicates how harnessing the redox activity of a nucleobase within an oligomer could have led to a primitive version of modern nucleotide cofactors. **Jason G. Underwood, Ph.D.**

New Target for Anti-tuberculosis Drug Development

Pyrazinamide (PZA) is a well-known frontline drug used in combination treatments for patients suffering from tuberculosis (TB). This drug has been shown to efficiently reduce treatment time from previously 9−12 months to 6 months. Although this nicotinamide analogue was first discovered more than 60 years ago, little is known about its target in *Mycobacterium tuberculosis*. However, the ability of PZA to target dormant *M. tuberculosis* bacilli specifically made it a highly desirable model drug that shortens the therapy. Now, Shi *et al.* (*Science* 2011, *333*, 1630−1632) identify the target of PZA and, in doing so, find a much-sought focus for anti-TB drug development.



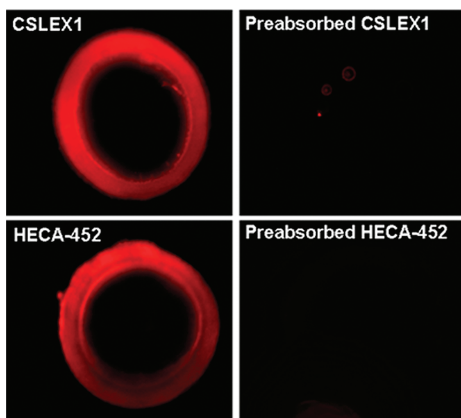
From Shi, W., *et al.*, *Science*, 2011, *333*, 1630. Reprinted with permission from AAAS.

PZA is a prodrug that is functional as an anti-TB compound following hydrolysis in *M. tuberculosis* cells to pyrazinoic acid (POA) by the enzyme, pyrazinamidase encoded by *pncA* gene. Using a combination of binding studies and mass spectrometry, the authors identified four possible targets. Of these four targets, the authors focused on ribosomal protein S1 (RpsA), a protein essential for the translation of proteins. RpsA is also involved in trans-translation, a process by which stalled ribosomes on mRNA are dislodged and replaced by transfer-mRNA (tmRNA). Specifically, RpsA binds tmRNA and subsequently complexes with other proteins involved in translation, *i.e.*, small protein B (SmpB) and elongation factor-Tu (EF-Tu) bound to GTP. This complex plays the essential role of removing stalled ribosomes and resuming translation. Overexpression of RpsA was found to increase resistance to PZA. The authors showed that POA bound directly to RpsA using an *in vitro* assay. The identification of RpsA as a target for persister drug PZA opens the door for developing more potent compounds against this target and

related trans-translation pathway for more effective eradication of TB. **Jitesh A. Soares, Ph.D.**

Elucidation of the Zona Pellucida

We all know that the initial step of human fertilization occurs when a sperm cell, or spermatozoan, binds to an egg cell, or ovum. Perhaps less well-known is that this binding event is mediated by the interaction between a protein on the sperm cell surface and carbohydrate moieties on glycoproteins present in the zona pellucida, which is the outer coating of the ovum. However, the challenges associated with obtaining human eggs for research purposes and the notorious complexities in the characterization of cell surface oligosaccharides have hindered the identification of the specific carbohydrates and proteins involved. Pang *et al.* (*Science* 2011, *333*, 1761−1764) now report that sperm binds to the zona pellucida through a well-known tetrasaccharide moiety called sialyl-Lewis^x.



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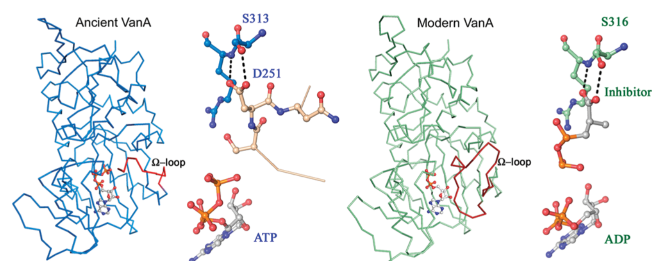
Nearly 20 years ago, evidence was presented that the major carbohydrate sequences presented on the human zona pellucida reacted with adhesion molecules known as the selectins. Selectins mediate cell adhesion during the initial stages of the inflammatory response, and sialyl-Lewis^x is their universal ligand. Now, mass spectrometry analyses demonstrated that sialyl-Lewis^x is the most abundant terminal oligosaccharide sequence present in the zona pellucida, and surprisingly it was present at significantly higher densities than those found in other cells. The interaction was characterized using the hemizona assay, in which nonliving human eggs are split into two equivalent hemispheres to provide an internal control for sperm binding. Sperm were prevented from binding to the zona pellucida in the presence of sialyl-Lewis^x-BSA or antisialyl-Lewis^x antibodies, and when solubilized zona pellucida was desialylated. In addition, fluorescently labeled sialyl-Lewis^x-BSA bound to sperm but not the hemizona. These findings are an exciting step forward in understanding the molecular underpinnings of human fertilization. Notably, since selectins are not expressed on the sperm surface, the hunt for the sperm protein that binds sialyl-Lewis^x continues. **Eva J. Gordon, Ph.D.**

Uncovering the Origins of Antibiotic Resistance

Less than a century ago, antibiotics were touted as wonder drugs, but almost immediately microbes emerged that were resistant to these compounds. As resistance has grown, questions have arisen about the origins of these genetic changes. Initially researchers thought that resistance mechanisms have developed in response to the use of antibiotics. More recently, studies of microbial genomes have found that resistance genes are more concentrated and more widely distributed than expected, suggesting that those changes might date back thousands rather than 100 years. Now D'Costa *et al.* (*Nature*, 2011, 477, 457−461) have found an ancient and diverse mix of antibiotic resistance genes in 30,000-year-old sediments from the Arctic. The results show that resistance genes arose long before the modern use of antibiotics.



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The researchers collected sediments from permafrost near Dawson City, Yukon Territory, an area where the age can be accurately dated and that has remained frozen during that period. Initially the researchers confirmed the presence of genetic material from plants and animals from the Pleistocene era such as ancient sage and grasses and mammoths and bison by amplifying DNA isolated from the sediments. They developed genetic assays to fish for specific types of resistance genes and found a range of sequences that match a diverse set of resistance mechanisms including genes that encode for β-lactamases and the tetM, a protein that blocks tetracycline antibiotics from binding to the ribosome.

They also pulled out *vanX*, one of the three genes (*vanHAX*) that work together to confer resistance to vancomycin, an antibiotic used against bacteria that are no longer susceptible to other drugs. With further PCR analysis, the researchers confirmed the presence of all three resistance genes. To demonstrate that the genes are functional, the open reading frames of these genes were cloned and expressed. The resulting proteins had activity that matched their modern counterparts, and a crystal structure of ancient VanA is similar to the modern enzyme.

Because new antibiotics will select for genetic resistance mechanisms that already exist within the genomes of microbes, this paper underscores the need for careful use of all antibiotics. **Sarah A. Webb, Ph.D.**

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