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Spotlight

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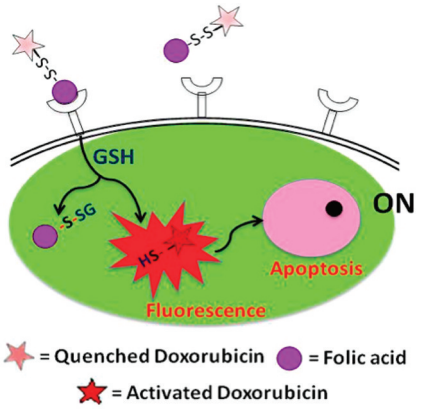
TRIFUNCTIONAL TARGETING

Targeted drug therapy refers to drugs that are capable of killing or otherwise inactivating diseased cells selectively over healthy cells, resulting in more effective treatment and fewer side effects. A promising strategy for developing targeting agents involves conjugation of a cytotoxic agent, such as a chemotherapy drug, to a targeting agent, such as a molecule that binds to a receptor that is overexpressed on the surface of abnormal cells relative to normal cells. To this end, Santra *et al.* (*J. Am. Chem. Soc.* 2011, *133*, 16680−16688) report the design and unique biological activity of a conjugate of the chemotherapy drug doxorubicin to the small molecule folate, which directs the conjugate to cancer cells overexpressing the folate receptor.



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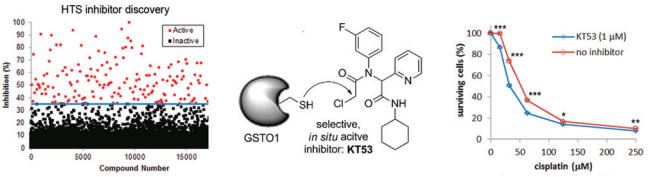
On the basis of previous studies suggesting that conjugation to the amine group of doxorubicin does not affect its biological activity, folate was covalently attached to this amine *via* a disulfide linker. It was anticipated that in addition to selectively binding to folate receptor-expressing cancer cells, the conjugate would be reduced upon entry into the reducing environment of the cytosol, and doxorubicin would be released. It was fortuitously discovered, however, that conjugation of doxorubicin to folate resulted in both a significant reduction in its cytotoxicity as well as quenching of its inherently fluorescent properties as an anthracycline derivative. Thus, the folate-doxorubicin conjugate functioned as a prodrug that was inactive and quenched until entering the cell cytoplasm. The authors propose that once inside the target cell the prodrug undergoes disulfide bond reduction, releasing doxorubicin and resulting in a concomitant increase in doxorubicin-associated fluorescence. In addition, upon entering the nucleus, doxorubicin exerts its cytotoxic DNA intercalating activity. This novel conjugate thus simultaneously functions in three important ways: a prodrug, a targeting agent, and an imaging agent, and points to an intriguing new strategy for the developing of similar trifunctional agents.

Eva J. Gordon, Ph.D.

ATTACKING THE RESISTANCE

Some cancer cells have a notorious talent for resisting the actions of chemotherapeutic drugs. Glutathione *S*-transferase omega 1 (GSTO1), a member of the glutathione *S*-transferase (GST) superfamily of enzymes, has been implicated in chemotherapeutic resistance. However, the lack of potent and selective GSTO1 inhibitors has hindered exploitation of this potential target for combating drug resistance. Tsuboi *et al.* (*J. Am. Chem. Soc.* 2011, *133*, 16605−16616) now report the discovery of novel GSTO1 inhibitors and demonstrate their efficacy in sensitizing otherwise resist cancer cells to traditional chemotherapeutic agents.



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The active site cysteine in GSTO1 distinguishes it from the majority of other GSTs, which utilize serine or tyrosine as their active site nucleophiles. Inhibitors specific for GSTO1 are thus likely to possess thiol-reactive moieties such as sulfonate esters and haloacetamides. A strategy called fluorescence polarization activity-based protein profiling ((fluopol)-ABPP) was used to identify such GSTO1 inhibitors. The 300,000+ public NIH small-molecule library was screened for the ability to block binding of a fluorescent GSTO1 activity-probe to the enzyme. From an initial ∼3200 hits, retesting and various secondary assays led to the selection of 10 compounds, 9 α-chloroacetamides and 1 α-aryl chloride, for selectivity testing and structure optimization. Ultimately, a compound referred to as KT53 was chosen for further testing in cells due to its superior potency, selectivity, and pharmacological properties. In addition, an alkyne analogue of KT53 called KT59 was synthesized so that click chemistry could be used to assess the presence of additional inhibitor-reactive proteins in cells. Notably, KT53 alone showed limited toxicity in cells, but when given to cancer cells in combination with the common chemotherapy drug cisplatin, the cells were significantly more sensitive to the cytotoxic effects of the drug. This study offers pharmacological evidence for the involvement of GSTO1 in chemotherapy resistance and delivers exciting lead compounds for future development.

Eva J. Gordon, Ph.D.

MAPPING CONSTRAINT

It is difficult to overstate the tremendous knowledge gained, both in our understanding of biology and disease as well in technological advances, from efforts over the past few decades to sequence the human genome. Many unexpected findings,

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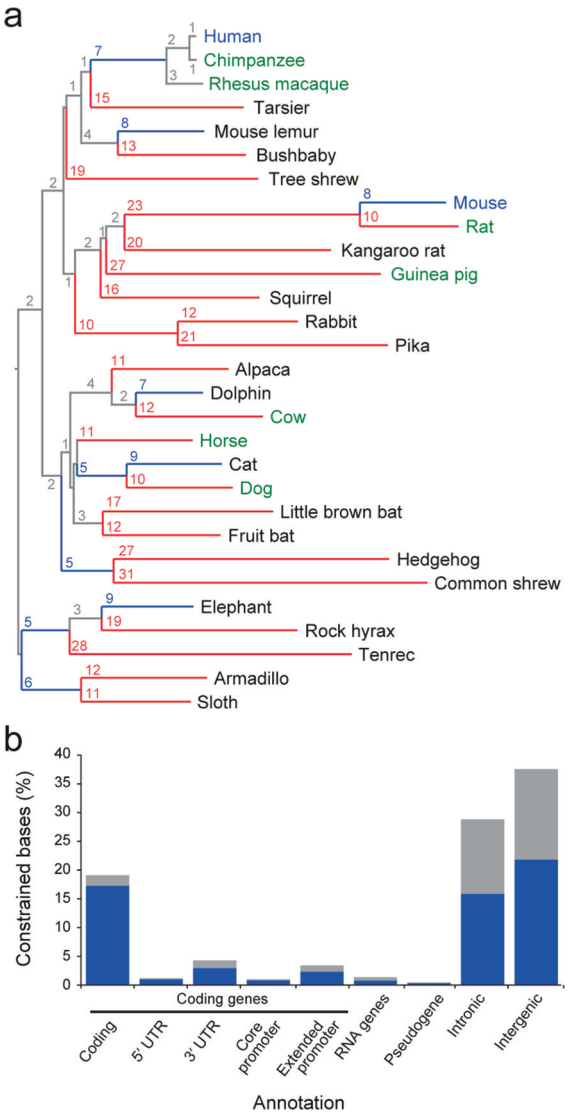
such as the presence of just over 20,000 protein-coding genes and the realization that the vast amount of noncoding DNA, once called "junk" DNA, likely plays critical functional roles in biology. Comparison of the human genome with the genomes of other mammals is a stimulating approach for deciphering the myriad aspects of the genome that remain enigmatic. Lindblad-Toh *et al.* (*Nature* advance online publication October 12, 2011; DOI: 10.1038/nature10530) take this approach in their report of the sequencing and comparative analysis of the genomes of 29 mammalian species spanning the four major mammalian clades, including mammals as diverse as humans, dolphins, sloths, shrews, and bats.



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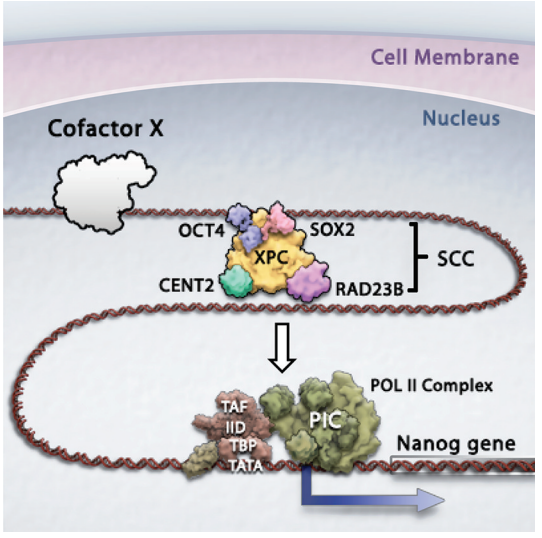
Identification and analysis of regions of mammalian genomes under evolutionary constraint, *i.e.*, those portions that have not changed over long periods of time, provide important clues into the genetic underpinnings of what makes mammals uniquely mammalian. This comparative analysis of the genomes of numerous mammals enabled the authors to generate a high-resolution map of the constrained elements in the human genome, of which over 3.5 million were identified. Moreover, they were able to ascribe potential functional classes to ~60% of the constrained bases. Among their many discoveries were the identification of new protein coding regions and previously undescribed RNA structures. Notably, they also find evidence for mobile element exaptation, which refers to features that may have evolved for one purpose but were co-opted at a later time for another function, and accelerated evolution in mammals. This impressive characterization of the human genome has broad implications for biology and medicine, including facilitating the identification of the genetic basis for disease.

Eva J. Gordon, Ph.D.

STEM CELLS GO IN FOR A REPAIR

Maintaining pluripotence, or the ability to differentiate into any cell type, is a key challenge for the pluripotent stem cell. This cellular program requires activation of a specific set of transcriptional events both to keep "stemness" factors switched on and to repress differentiation events. Three key transcription factors, Oct4, Sox2, and Nanog, are all critical for pluripotency and actually act on one another in the cell. Sox2 and Oct4 are both required to activate stem cell expression of Nanog and do so in the classical manner with both factors binding upstream of the Nanog transcription start site. Since these factors are not sufficient for turning on Nanog in other cell types, Fong *et al.* (*Cell* 2011, *147*, 120–131) recently set out to identify additional factors that make stem cells primed for Nanog activation.



Reprinted from *Cell*, *147*, Fong, Y. W. *et al.*, A DNA repair complex functions as an oct4/sox2 coactivator in embryonic stem cells, 120–131. Copyright 2011, with permission from Elsevier.

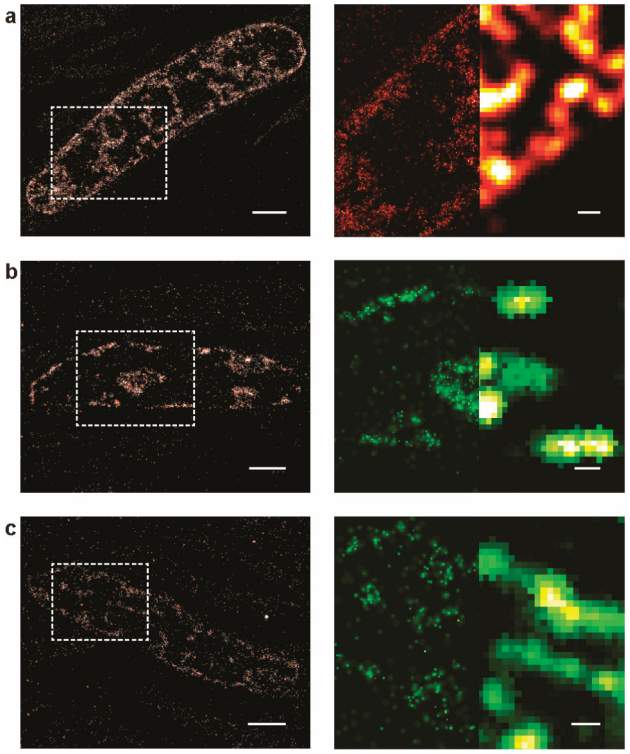
Using a minimalist Nanog promoter, the researchers could recapitulate the Sox2/Oct4-dependent activation *in vitro*. They then used a biochemical fractionation and complementation approach to hunt for the mystery activation factor or factors from a pluripotent cell nuclear extract. After eight chromatographic steps, an activation fraction termed the SCC, or stem cell coactivator, showed extremely robust activity in the *in vitro* assay and was subjected to mass spectrometry analysis to identify the components. In a surprising turn of events, the SCC fraction contained three members of the nucleotide excision repair (NER) complex, XPC, RAD23B and CETN2. The researchers went on to validate this curious finding by first showing that members of this DNA repair complex show similar expression timing to

known pluripotency proteins. Then, they produced a recombinant version of the SCC in insect cells to demonstrate that these three factors are sufficient to coactivate the Nanog promoter in concert with Sox2 and Oct4. Finally, they took the wisdom gained from the *in vitro* assay and returned to the *in vivo* environment. There, they showed that knocking down members of this complex interfered with stem cell maintenance and the ability to produce induced pluripotent stem (iPS) cells. They also showed that this complex is found at most of the same loci in the genome that Oct4 and Sox2 occupy in stem cells, indicating that DNA repair and gene expression are collaborating partners in preserving the delicate state of pluripotency.

Jason G. Underwood, Ph.D.

BETTER MOLECULES FOR OBSERVING CELLS

Biology researchers continue to look for reliable ways to visualize the choreography of chemical interactions within living systems. The available tools include a variety of photoactivatable molecules that can be switched on and off in response to specific wavelengths of light. When attached to biological molecules, researchers can use super-resolution imaging techniques such as photoactivated localization microscopy (PALM) to pinpoint the position of individual molecules in living cells.



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Photoactivatable "caged" fluorophores can be incredibly difficult and costly to synthesize, but now Wysocki *et al.* (*Angew. Chem., Int. Ed.*, 2011, DOI: 10.1002/anie.201104571) report a general method for producing xanthenes-based caged dyes. In response to light, these molecules convert from a nonfluorescent lactone to the fluorescent quinoid forms. This central chemical functionality also makes these molecules especially difficult to synthesize, requiring harsh conditions and producing multiple side products. Wysocki *et al.* avoided this problem by using catalytic hydrogenation to reduce these xanthenes dyes to their "leuco" derivatives. These intermediates are more reactive, allowing the researchers to add functional groups using milder conditions. As a final synthetic step, the dyes were reoxidized using DDQ, which also liberated a carboxyl group for bioconjugation. The researchers used these techniques to synthesize Q-rhodamine, a promising but difficult to synthesize PALM probe, rhodamine 110, and 2′,7′-difluorofluorescein.

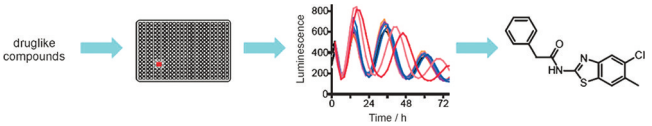
Biotin adducts of these dyes were chemically stable and their fluorescent efficiency met or exceeded that of mEos2-biotin, a molecule routinely used in PALM studies. The researchers then set up a super-resolution microscopy experiment. First, they converted the appended carboxylate on the dyes to and azide and used copper-based "click chemistry" to link those dyes to alkynyl nucleobases. Using all 3 dyes, they successfully imaged the position of newly synthesized cellular DNA, the first time that this biopolymer has been imaged with this technique within a cell.

This clever and simple synthetic technique opens up the possibility of producing a range of photoactivatable synthetic dyes that respond to different wavelengths of light. Because these dyes can now be easily synthesized in any organic chemistry laboratory, super-resolution microscopy will be accessible to an increasing number of researchers.

Sarah A. Webb, Ph.D.

ALTERING TIME

Many species of flora and fauna have been shown to possess internal circadian clocks that manage the biological rhythms of major biological processes. These biological clocks play a role in many important aspects of mammalian life such as sleep, physical activity, immune function, *etc.* Disturbing normal circadian rhythm has been linked to several pathologies such as sleep disorders, cardiovascular disease, and cancer. The identification of compounds that can moderate this internal clock is therefore important to developing new therapies for associated disorders. Lee *et al.* (*Angew. Chem., Int. Ed.*, 2011, DOI: 10.1002/anie.201103915) report the identification of one such compound that can modulate circadian clocks.



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The authors used a high-throughput circadian assay system, which they had previously developed, to screen 500,000 compounds for the ability to prolong the circadian period in human U2OS cells. The screen identified numerous different chemical scaffolds. A benzothiazole derivative, LH846, showed the greatest time lengthening (10 h) characteristics. To identify the enzyme target of this compound, LH846 was attached to a solid support *via* a di(ethylene glycol) linker that was used for pull-down assays. Using a combination of gel electrophoresis, affinity chromatography, Western blotting, and mass spectrometry, the LH846 target was shown to be casein kinase 1 delta

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(CKIδ), a known modulator of the circadian clock. Additionally, LH846 was shown to inhibit CKIδ-dependent phosphorylation and degradation of PER1, a cellular event previously shown to play an important role in regulating cellular rhythm. Thus, this study provides researchers with a new tool for the specific inhibition of a kinase that plays an important role in biological rhythms.

Jitesh A. Soares, Ph.D.

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