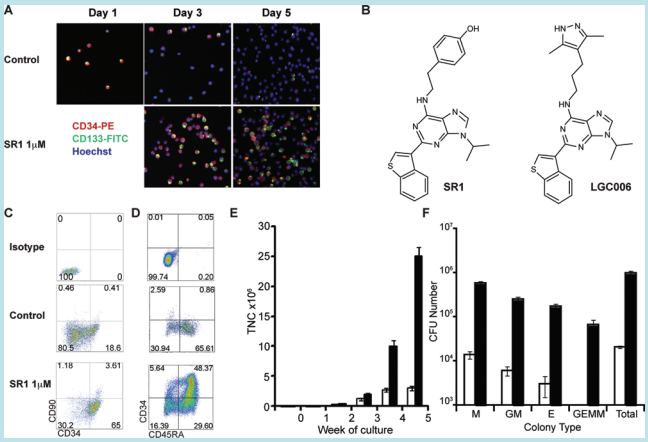
Spotlight

Out for Blood Cells



From Boitano, A. E., *et al.*, *Science*, 2010, *329*, 1345. Reprinted with permission from AAAS.

Stem cell transplantation provides a disease-free supply of blood cells to help the body fight a variety of hematological disorders and cancers, such as certain anemias, leukemias, and lymphomas. Hematopoietic stem cells (HSCs), which can differentiate into all blood cell types, reside in the bone marrow and to some extent in the peripheral blood, but gaining access to adequate quantities of these cells for transplantation can be a significant challenge. Treatment with the growth factor cytokine granulocyte colony-stimulating factor (G-CSF) is a common method for mobilizing hematopoietic stem and progenitor cells (HSPCs) from bone marrow to peripheral blood, where they can be collected. However, this process is not always successful, and the pathways involved are not completely understood. The expansion of HSCs in cell culture is a complementary and promising approach for obtaining a sufficient supply of HSCs, but culture conditions that support HSC expansion are not well-defined. Now, Ryan *et al.* (*Nat. Med.* 2010, *16*, 1141−1146) and Boitano *et al.* (*Science* 2010, *329*, 1345−1348) reveal exciting insights into the mechanisms underlying HSC mobilization and expansion.

To explore the mechanisms involved in G-CSF-dependent mobilization of HSCs, Ryan *et al.* employed a forward genetic approach in mice. Specifically, use of a congenic mouse model facilitated identification of a 5 MBp region of chromosome 11 that conferred enhanced mobilization of HSPCs, and gene chip expression analysis and quantitative real-time RT-PCR experiments revealed that the enhanced mobilization correlated with a reduction in *Egfr* gene expression in hematopoietic progenitor cells (HPCs) isolated from bone marrow. Further, increased mobilization was observed in mice that either possessed a genetic mutation that reduced EGFR activity or that were treated with erlotinib, a small molecule inhibitor of EGFR activity. Investigation into the mechanism of this effect revealed the involvement of Cdc42, a downstream target of EGFR known to be involved in the migration and adhesion of HSPCs. Specifically, genetic reduction in Cdc42 activity also resulted in enhanced mobilization, suggesting that the mobilization process is controlled in part by EGFR signaling through Cdc42. These findings point to EGFR inhibitors as potential agents for enhancing HSPC mobilization for stem cell transplantation in the clinic.

Under defined cell culture conditions, HSCs can be coaxed to proliferate, which offers another potential source of HSCs for transplantation. However, the proliferation is accompanied by differentiation, which results in a loss of the precious multipotent potential of the cells. In an effort to find conditions that promote expansion without differentiation, Boitano *et al.* used primary human HSCs and confocal microscopy to screen 100,000 heterocycles for compounds that increased the number of cells expressing CD34 and CD133, cell surface markers present on HSCs and HPCs that are lost upon differentiation. A purine derivative referred to as SR1 was found to promote an increase in the number of $CD34^+$ and $CD133^+$ cells. Engraftment studies, which measure the ability of the transplanted cells to generate new blood cells, using immunodeficient mice demonstrated that culture with SR1 leads to substantially more HSCs capable of differentiating into multiple blood cell types compared to uncultured $CD34^+$ cells. Transcriptional profiling experiments pointed to the aryl hydrocarbon receptor (AHR) transcription factor as a likely target for SR1. Indeed, further investigation revealed that SR1 inhibits AHR through a direct binding interaction.

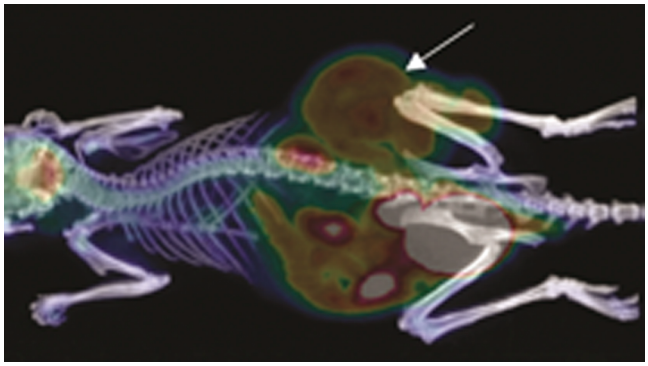
These studies probing the mechanisms that dictate HSC mobilization and proliferation have elucidated two exciting new approaches for increasing access to HSCs for stem cell transplantation, and highlight the power of chemical biological approaches for exploring hematopoietic stem cell biology. **Eva J. Gordon, Ph.D.**

Published online November 19, 2010 • 10.1021/cb100343x

Spotlight

Inhibiting Epigenetic Readers

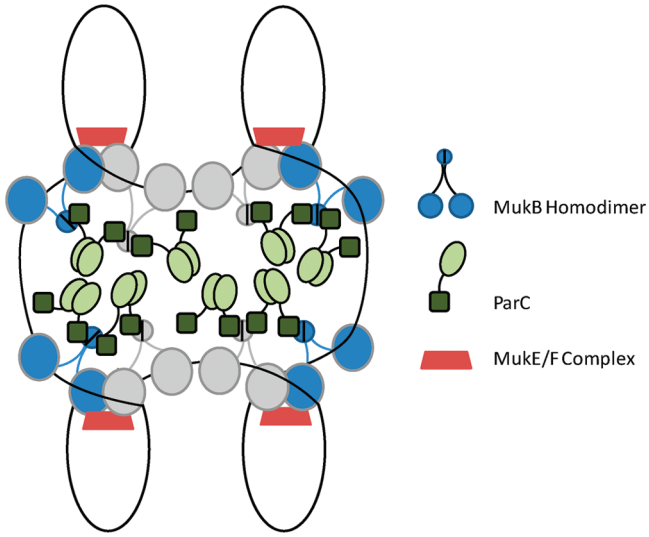
Epigenetic marks, such as methylation of DNA and acetylation of histone lysine residues, are critical determinants of gene regulation. Though intense research has been conducted on the enzymes that manufacture these marks, much less is known about the proteins that recognize them. Bromodomains are conserved α-helix-containing structures present in dozens of diverse human proteins that recognize acetyl-lysine groups. Recent evidence has linked the BET family of bromodomain-containing proteins to certain cancers, including chronic lymphocytic leukemia and human squamous carcinoma, hinting at the possible anticancer potential of inhibiting bromodomain activity. Now, Filippakopoulos *et al.* (*Nature* advance online publication September 24, 2010; DOI: 10.1038/nature09504) describe the identification and biological characterization of a small molecule inhibitor of the BET family member BRD4.



Based on patent literature precedence suggesting that thienodiazepines bind BRD4, a novel thieno-triazolo-1,4-diazepine called JQ1 was synthesized and evaluated for BRD4 binding and biological activity. First, three binding assays (differential scanning fluorimetry, isothermal titration calorimetry, and a luminescence proximity homogeneous assay) were used to demonstrate that (+)-JQ1 is a potent and selective inhibitor of the BET family that competes for binding with acetylated lysine. Next, determination of the cocrystal structure of BRD4 with (+)-JQ1, along with molecular dynamics simulations, confirmed that (+)-JQ1 binds to the acetylated lysine binding site of BRD4, and cellular studies using fluorescence recovery after photobleaching experiments indicated that (+)-JQ1 displaces BRD4 from nuclear chromatin. Furthermore, when exposed to JQ1, cells derived from squamous carcinoma patients underwent terminal differentiation and apoptosis. Finally, xenograft models of NMC in mice treated with JQ1 exhibited reduced tumor growth and minimal adverse effects, establishing the anticancer activity of BRD4 inhibition. This study exposes inhibition of epigenetic readers such as BRD4 as an exciting, relatively untapped area in the manipulation of epigenetic regulation for therapeutic benefit. **Eva J. Gordon, Ph.D.**

A Partner for MukB

Cell division requires the precise orchestration of numerous complex events, including chromosome replication, condensation, and segregation. Though intensely investigated in eukaryotic organisms, these processes are much less well-defined in prokaryotes. It is known, however, that a member of the structural maintenance of chromosomes protein family called MukB is a key player in prokaryotic chromosomal reorganization. Given the complexity of the process, MukB likely cooperates with several protein partners, but few have been identified. Now, Li *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* published online October 4, 2010; DOI: 10.1073/pnas.1008678107) report that MukB interacts with a component of topoisomerase IV (Topo IV), another important modulator of chromosome structure during cell division.



Affinity purification and mass spectrometry were initially used to find proteins that bind to a truncated version of MukB. These studies led to the identification of ParC, the DNA binding and cleavage subunit of Topo IV. Additional immunoprecipitation experiments, along with pull-down and isothermal titration calorimetry studies, demonstrated that MukB and ParC indeed interact directly and revealed insights into the location of the interaction. Next, DNA relaxation and decatenation assays and alanine scanning mutagenesis were employed to investigate the functional significance of the MukB-ParC interaction. It was shown that MukB stimulates and enhances the rate of both the DNA relaxation and decatenation activities performed by Topo IV. In addition, aspartate 692 of MukB was identified as critical for the interaction between MukB and ParC. Specifically, a MukB mutant in which the aspartate was replaced with alanine exhibited decreased binding to ParC and was incapable of rescuing the growth of a temperature-sensitive *mukB*$^{-}$ strain. Notably, in a related paper by Hayama and Marians (*Proc. Natl. Acad.*

Sci. U.S.A. published online August, 9, 2010; DOI: 10.1073/pnas.1008140107), a ParC variant was identified that lacked that ability to interact with MukB. While this mutant was still fully active as a topoisomerase, the ability of MukB to stimulate its relaxation activity was abolished, further substantiating the importance of the physical interaction between MukB and ParC for their functional relationship. These studies identify ParC as an important functional partner for MukB and offer much needed insight into the mechanisms underlying prokaryotic chromosomal reorganization processes. **Eva J. Gordon, Ph.D.**

Hormones Unveil a New Strategy

The jasmonates are plant hormones that play critical roles in growth, development and physiology by acting as potent regulators of many downstream processes. While some hormones bind to their receptors and go on to upregulate transcription directly, the jasmonates function by regulated degradation of key transcriptional repressors. Jasmonates are activated by conjugation with the natural amino acid, isoleucine, to form the active hormone, JA-Ile. Previous studies in *Arabidopsis* indicated that COI1, a component of a ubiquitin ligase complex, was involved in sensing the JA-Ile and marking JAZ-family transcriptional repressors for degradation. To better understand the pathway, a new study (Sheard *et al. Nature* advanced online publication October 6, 2010; DOI: 10.1038/nature09430) used a powerful combination of binding studies and structural biology to uncover the molecular mechanism of how plants sense jasmonates.

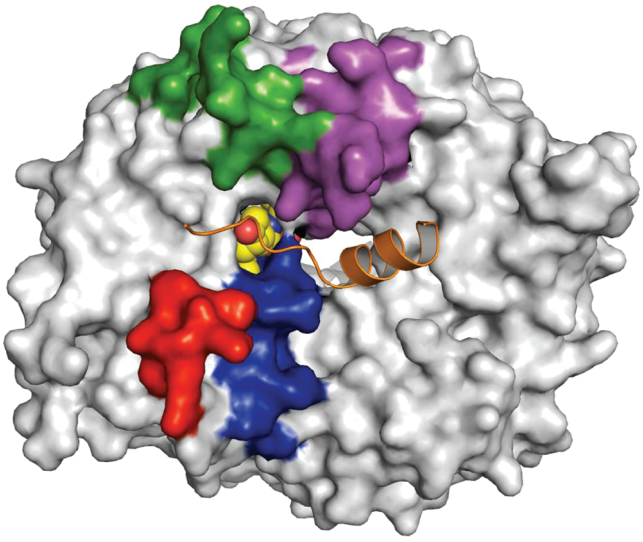
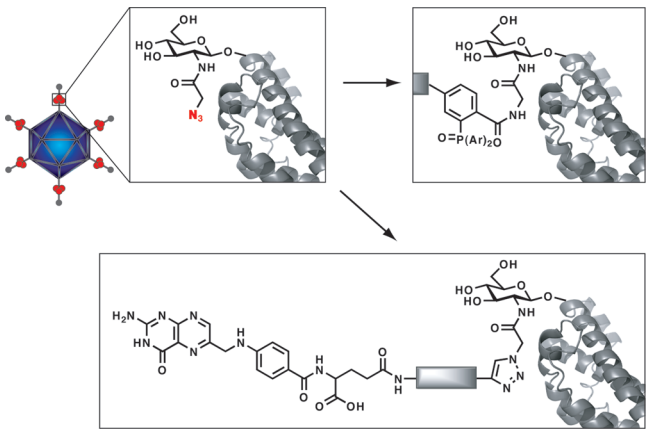


Image courtesy of Laura Sheard.

To learn more about how jasmonate is specifically recognized, the authors used a natural toxin from *Pseudomonas* bacteria, coronatine, previously known to target the jasmonate pathway. With just recombinant COI1 or a JAZ protein present, coronatine did not bind, but when both were present, the toxin bound with high affinity. This indicated that the same protein that will soon be marked for degradation, JAZ, is also a coreceptor for the jasmonate hormone. This assay was further used to determine which amino acids of JAZ are important for hormone binding and elucidation of a consensus degron peptide. With this information in hand, the authors turned to crystallography to determine the structure of the COI1 complexed with the ubiquitin ligase, ASK1, the JAZ degron peptide, and either the natural hormone JA-Ile or the toxin coronatine. The results showed how the degron peptide functions in the complex by docking to COI1 via a conserved α-helix while simultaneously trapping the hormone into its binding pocket via degron peptide's loop region. Interestingly, an additional component critical for sensing jasmonate was uncovered while building the crystallographic model. Mass spectrometry data for the COI1-ASK1 complex showed two peaks with a difference of ~568 Da. After an impressive chromatographic scheme, the copurifying molecule of interest, inositol pentakisphosphate, was identified. The authors went on to demonstrate that it is a *bona fide* cofactor by stripping away the inositol pentakisphosphate and showing that COI1-JAZ1 could no longer bind to coronatine. This study reveals the mechanism of a critical plant hormone but also shows how inositol phosphates can play unexpected roles in cellular sensing pathways. **Jason G. Underwood, Ph.D.**

Gene Delivery in a Sweet Package

Although viruses have evolved the ability to transfer genes under defined circumstances, engineering these infective agents to deliver a broader array of gene products has proved challenging. The inability to customize the chemistry on the surface of the virus particle has made it difficult to tailor infective agents to specific targets. Now Banerjee *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 13615−13617) report a new strategy that decorates a viral surface with azido-sugars, which serve as a chemical canvas for attaching a variety of other functional molecules. The researchers then demonstrate the delivery of genes with a modified virus to mouse cancer cells.



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The fiber protein of human adenovirus 5 (Ad5) contains a serine residue modified with a carbohydrate (*O*-GlcNAc) at the edge of the virus, a prime location for tweaking the targeting of the virus. As the viral particles were being produced in cells, the researchers added azido-glucose and galactose precursors to the medium to incorporate those carbohydrates on the surface. With azides incorporated at approximately half of these exterior carbohydrates, the researchers then used copper-mediated click chemistry to efficiently link these carbohydrates to other functional molecules via an alkyne tether.

To test targeted gene delivery to cancer cells, the researchers modified available azides on the surface of Ad5 with tethered folate molecules: folate receptors are overexpressed on cancer cells but are comparatively rare on normal cells. Within the virus, they replaced a section of the genome with a transgene encoding green fluorescent protein (GFP). After 24 h, cells infected with the modified virus showed GFP expression up to 4-fold higher than those infected with Ad5 with a normal surface. Infections carried out with folate within the media inhibit the viral transfection in a dose dependent manner. This clever targeting strategy is highly specific and adaptable to other viruses with exterior glycoproteins, including retroviruses and lentiviruses. **Sarah A. Webb, Ph.D.**