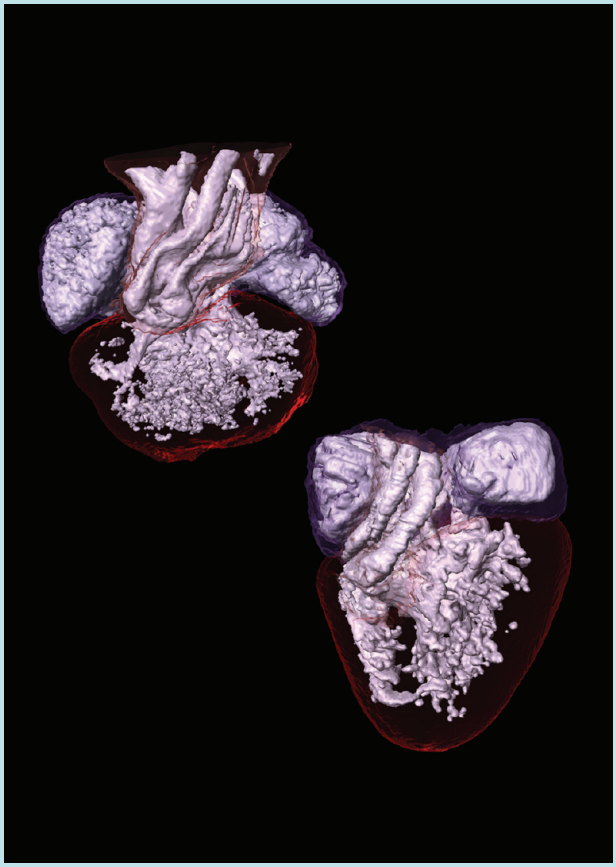
Spotlight

Uncovering the Chamber Secret



Reprinted by permission from Macmillan Publishers Ltd: *Nature.*, Koshiba-Takeuchi *et al.*, *461*, 95−98, copyright 2009.

The circulatory system has long remained a mystery within the evolutionary tree of animals. Cold-blooded animals like amphibians are powered by a three-chambered heart, while the warm-blooded terrestrial mammals and birds have gained an extra wall to create a full four chambers. This ancient adaptation meant more efficient exchange of oxygen, a key ingredient in the diversity of mammals, ranging from fast running cheetahs to deep diving whales. Adding to the mix are reptiles which act as a partial intermediate between the groups; non-crocodile reptiles have three chambers, but they have varying degrees of shunting *via* a primitive interventricular septum between the ventricles. Koshiba-Takeuchi *et al.* (*Nature* 2009, *461*, 95−98) compared the three-chambered heart of two reptiles to the four-chambered hearts of the mouse and chicken.

The group focused on the transcription factor, Tbx5, as this was previously implicated in heart patterning in multiple species and a loss of expression caused a defect in heart formation in mammals. In the chicken embryonic heart, Tbx5 is expressed early and in the cells that will eventually become the left ventricle. In contrast, developing reptile hearts from the turtle and the anole, a type of lizard, both showed Tbx5 expression throughout the developing organ. Interestingly, at a later stage in heart development, the turtle and anole started to show differences in expression. The turtle heart, which would eventually produce a more substantial interventricular septum than the anole, displayed a shifted pattern of Tbx5 expression to favor the left ventricle, while the anole Tbx5 levels stayed constant across the heart. But could this sharp patterning of one transcription factor be responsible for building a wall between the ventricles? To test this hypothesis, the researchers used a mouse model, producing a conditional allele of Tbx5 to see what effect this might have on ventricle formation. Remarkably, ablating Tbx5 expression in the left ventricle, or expanding its expression to mimic the reptile pattern, led to a developing heart resembling the reptiles' with one large ventricle. This study represents a fascinating molecular answer to an age-old anatomical question. **Jason G. Underwood, Ph.D.**

The Acetylation Nation Revealed

The role of reversible post-translational modifications (PTMs) in connecting chromatin biology to gene regulation continues to be a swirling brew of hot topics. Areas of recent interest are the acetylation of proteins by histone acetyltransferases (HATs) and the removal of this mark by histone deacetylases (HDACs). Other PTMs like phosphorylation and methylation have far-reaching roles in nearly all cellular processes, but the role of acetylation has remained poorly understood due to technical limitations. Now, a new methodology (Choudhary *et al. Science* 2009, *325*, 834−840) emerges that enriches for acetylated proteins and then uses quantitative mass spectrometry to identify and measure what the authors term the "acetylome", a peptide inventory of acetylation sites in several human cell lines.

The proof-of-principal came from the histones and the changes induced by HDAC inhibitors, while the proteins unrelated to chroma-

Published online October 16, 2009 • 10.1021/cb900237f CCC: $40.75

tin demonstrate that acetylation probably tweaks nearly every complex process in the cell. In the cytoplasm, dynamic macromolecular machines like the ribosome and actin cytoskeleton complexes were found to contain dozens of target sites for acetylation. Bridging the gap with the nucleus were many of the nuclear pore complex proteins and their cargo. Finally, back in the nucleus, hundreds of proteins other than the known chromatin targets were uncovered, and these spanned nearly every functional role. Proteins involved in DNA repair, RNA splicing, and control of the cell cycle all receive lysine acetylation marks. So, could reversible acetylation play regulatory roles in events like alternative splicing or trafficking of proteins and RNAs within the cell? Phylogenetic conservation scores and positioning for the target lysines argue that the answer is probably yes. This modification is ancient and the modified amino acids appear more often in conserved regions with predicted protein secondary structure. This study not only displays the power of an elegant new technique, but also defines a new area for exploration into how acetylation tinkers with cellular metabolism. **Jason G. Underwood, Ph.D.**

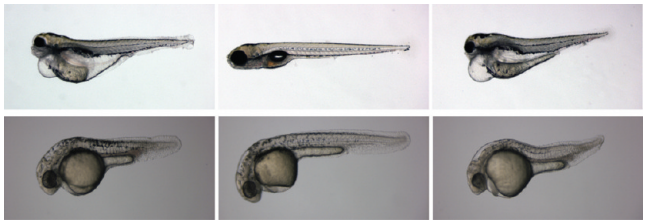
Technology Reads Another Man

Recent years have seen a bloom of new technology for reading nucleic acids in a massive parallel fashion. As enormous data sets emerge from what are being called the "next generation" sequencing platforms of companies like ABI, Illumina, and 454, it is clear that the era of personalized genomics is quickly becoming a more affordable reality. Now, a group has taken a test drive with an even newer Helicos sequencing platform with a difficult benchmark: a complete human genome (Pushkarev *et al. Nat. Biotechnol.* 2009, *27*, 847−852).

The machine performs single molecule sequencing *via* polymerization of fluorescent nucleotides in a stepwise fashion under the watchful eye of a sensitive microscope. Like other next generation technologies, the single-molecule machine can only read small 30- to 50-base fragments of DNA, so the assembly of a complete genome can only come from sequencing huge numbers of short reads and letting a computer churn at the data. The result was a complete genome at a cost that is several orders of magnitude cheaper than the first genomes published just 8 years ago. But how does the data compare? It seems to be a mixed bag, but with promise for the future. About 90% of the genome sequence was determined; this number is similar to the original genome publications and is largely due to repetitive regions that are not easily sorted out, especially with short reads. Every base was covered approximately 28 times, an essential redundancy since the error rate appears somewhat higher than for other platforms. The new platform does well at calling single nucleotide polymorphisms (SNPs), an essential benchmark since personalized genomics will require that platforms reliably find these divergences from a reference genome. Overall, this study shows a flavor of what is to come. The field is ripe with competition, and there is a sense of excited anticipation for the data that lie ahead. **Jason G. Underwood, Ph.D.**

Shining a Light on Embryo Development

Complex molecular programs regulate the development of an embryo into an organism. However, few chemical tools allow scientists to selectively tinker with gene expression patterns within an embryo to better understand the complex developmental program inside these cells. Now researchers have designed photoactivatable caged oligonucleotide analogues that they can use with an optically transparent organism, the zebrafish, to study these molecular mechanisms.



Ouyang *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 13255−13269) inhibited gene expression with morpholinos—analogues of nucleic acid polymers that replace ribose sugars and phosphodiesters with morpholine groups linked by phosphoramidates. To control the activity of these molecules, the researchers designed the molecules as inactivated hairpins called caged morpholinos (cMOs), in which the antisense morpholino sequence is connected to an inhibitory oligomer *via* a photocleavable linker based on a dimethoxynitrobenzyl (DMNB) group. By synthesizing a series of molecules of varying inhibitor lengths and hairpin configurations that targeted the *ntla* transcription factor in zebrafish embryos, the researchers optimized the probes to find inhibitory molecules that they could switch on using UV light. Disrupting *ntla* produces embryos that lack a notochord, with both a shortened posterior and an altered morphology. Using a combination of biophysical *in vitro* assays and profiles of *in vivo* activity of *ntla* cMOs, the researchers developed a general thermodynamic model of cMO activity based on a three-state equilibrium. The researchers then used cMOs to successfully target three other zebrafish genes: *heg*, *flh*, and *etv2*.

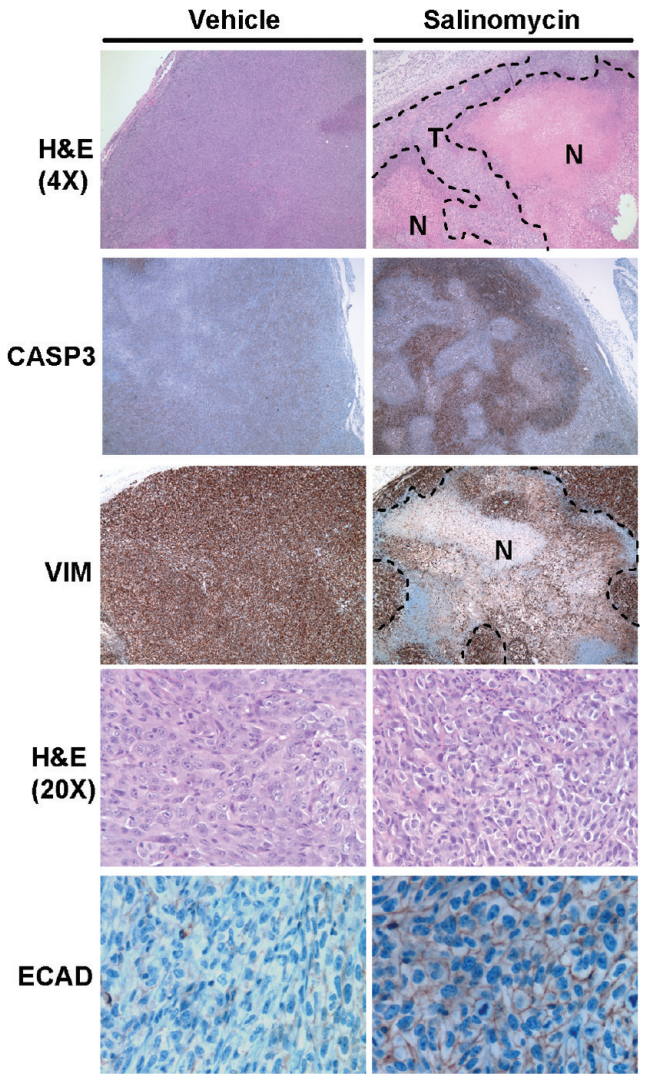
Because the UV light used to cleave the DMNB linker can damage DNA, the researchers also developed a new photocleavable linker for cMOs based on the bromohydroxyquinoline (BHQ) group. Such groups are activated by a two-photon irradiation at wavelengths above 700 nm. The research shows the power of chemistry to investigate developmental biology and offers design principles for

the development of other antisense reagents to probe these systems. **Sarah A. Webb, Ph.D.**

Targeting the Ugly Stepsister

The excitement around stem cells "stems" from their ability to differentiate into all cell types within a specific tissue or organism. Cancer stem cells (CSCs), which could perhaps be thought of as the ugly stepsisters of normal stem cells, can differentiate into all cell types within a tumor, intimating a unique accountability among tumor cells for their growth, metastasis, and recurrence. The broad resistance of CSCs to chemotherapeutic agents has hinted that they may be inherently impervious to drug treatment. Moreover, CSCs are often relatively rare within tumors and are not stable in cell culture, making searching for drugs that selectively target CSCs extraordinarily difficult. Now, Gupta *et al.* (*Cell* 2009, *138*, 645−659) report the development of a high-throughput screen to identify small molecules capable of selectively inhibiting CSCs.

A key to the success of the screen was to gain access to sufficient quantities of CSC-like cells to screen against. This was achieved by inducing non-tumorigenic immortalized mammary epithelial cells to pass through an epithelial-mesenchymal transition (EMT), which produces an enrichment of cells with stem-like properties and a drug resistance profile similar to that of CSCs. Of 16,000 compounds screened, 32 compounds were found to selectivity target cells that had undergone an EMT over cells that had not. When the compounds were tested against the corresponding tumorigenic cells, salinomycin, a potassium ionophore, emerged as a selective inhibitor. Strikingly, in *in vitro* experiments, salinomycin decreased the proportion of CSC cells by >2 orders of magnitude relative to the common breast cancer drug paclitaxel. Importantly, salinomycin treatment also inhibits breast tumor growth in mice. Subsequent gene expression experiments indicated that salinomycin induces a loss of breast CSC gene expression, offering a molecular rationale for the observed effects. This proof-of-concept demonstration that CSCs can be selectively targeted outlines an exciting new approach for cancer drug discovery efforts. **Eva J. Gordon, Ph.D.**

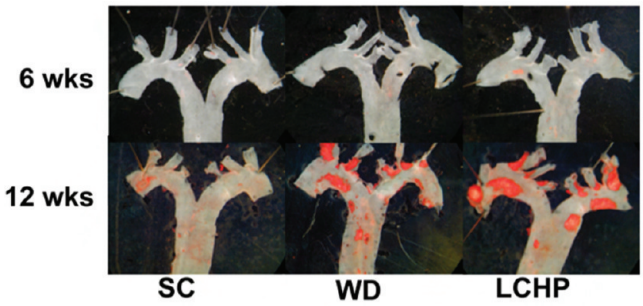


Reprinted from *Cell*, *138*, Gupta, P. B., *et al.*, Identification of Selective Inhibitors of Cancer Stem Cells by High-Throughput Screening, 645−659, Copyright 2009, with permission from Elsevier.

Cardiovascular Effects of the Low-Carb Craze

Over the past decade or so, low-carbohydrate, high-protein diets have become quite the diet craze. Promising to shed pounds with supposedly (and somewhat counterintuitively) few adverse health effects, these diets have been embraced from the slightly pudgy to the overtly obese. And, while it does appear that low-carb diets do not significantly affect serum markers of cardiovascular health, the long-term effects of these diets on the heart are unknown. Foo *et al.* (*Proc. Natl. Acad. Sci.*, 2009 *106,* 15418−15423) explore the cardiovascular effects of a low-carbohydrate, high-protein diet in mouse models of atherosclerosis and ischemia-induced neovascularization.

Three diets were compared in these studies: a low-carbohydrate, high-protein diet (LCHP); a standard chow diet (SC), which has more carbohydrate but less fat and protein than the LCHP diet; and the Western diet (WD), which has similar fat intake as the LCHP diet but more carbohydrate and less protein. Perhaps not surprisingly, mice on the LCHP diet did gain less weight than their SC and WD fed counterparts. In addition, standard cardiac risk markers, including cholesterol, glucose, inflammatory mediators, and oxidative stress levels, were not significantly altered in LCHP mice. However, LCHP mice did exhibit significantly increased atherosclerosis than WD mice



Foo, S. Y., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *106*, 15418–15423. Copyright 2009 National Academy of Sciences, U.S.A.

and were less able to generate new blood vessels in response to ischemic injury. Examination of the dietary effects on endothelial progenitor cells (EPCs), which have been implicated in vascular repair, indicated a stark reduction in EPC levels in both peripheral blood and bone marrow. Moreover, EPCs from LCHP mice exhibited decreased levels of phosphorylated Akt, a protein kinase important for EPC function. These results illuminate alternate pathways by which diet can affect cardiac health and justify closer examination of the use of LCHP diets for weight loss purposes. **Eva J. Gordon, Ph.D.**