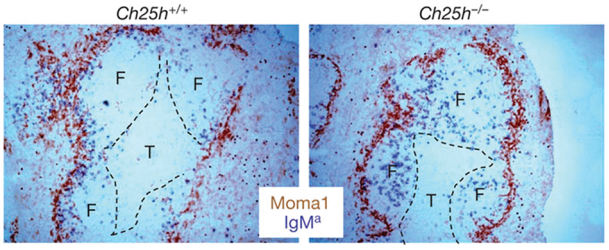
ACS chemical biology

SPOTLIGHT

pubs.acs.org/acschemicalbiology

Oxysterols Attract

Epstein–Barr virus-induced gene 2 (EBI2), named for its increased expression upon infection with Epstein–Barr virus, is a G-protein-coupled receptor involved in the immune response and implicated in autoimmune disorders. The receptor is known to direct B cells, key players in the adaptive immune response, to organs of the immune system such as the lymph nodes and the spleen and in facilitating T-cell-dependent antibody production. Oxysterols are oxidized derivatives of cholesterol that play important roles in the immune and inflammatory response as well as in cholesterol metabolism. Though the involvement of both EIB2 and oxysterols in the immune response is well established, two new reports, Liu *et al.* (*Nature* advance online publication July 27, 2011; DOI: 10.1038/nature10226) and Hannedouche *et al.* (*Nature* advance online publication July 27, 2011; DOI: 10.1038/nature10280), now illuminate a previously unknown connection between these biomolecules.



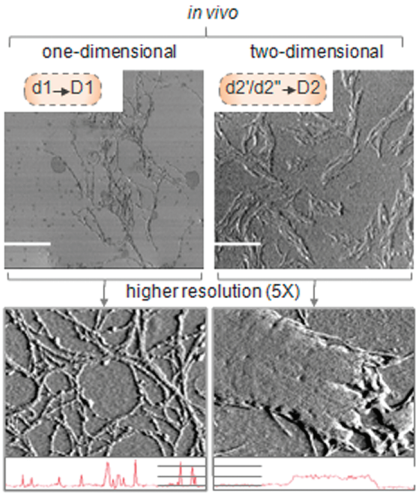
Reprinted by permission from Macmillan Publishers Ltd: *Nature*, advance online publication, 27 July 2011, DOI: 10.1038/nature10280

In a report by Hannedouche *et al.*, EBI2 activators were discovered by screening a liver extract from a sheep model of inflammatory disease for compounds capable of inducing intracellular calcium release. Extensive mass spectrometry and nuclear magnetic resonance analysis led to the identification of 7α,25-dihydroxycholesterol (7α,25-OHC) as the active compound. Structural and pharmacological characterizations demonstrated specific binding to EBI2 and not to various nuclear hormone receptors, to which oxysterols are known to bind. Similarly, in a related study by Liu *et al.*, oxysterols isolated from porcine spleen were identified as EBI2 ligands, and in agreement with Hannedouche *el al.*, 7α,25-OHC was the most active compound. In both studies, 7α,25-OHC was found to function as a chemoattractant *in vitro*, inducing the migration of EBI2-expressing cells. In addition, cells from mice lacking expression of either EBI2 or CH25H, an enzyme required for the biosynthesis of 7α,25-OHC, did not possess chemoattractant activity. The chemoattractant ability of 7α,25-OHC was also demonstrated *in vivo*. Activated B cells were prevented from localizing properly within lymphoid organs in EBI2-deficient mice, in CH25H-deficient mice, in mice in which normal B cells were desensitized by pretreatment with 7α,25-OHC, and in mice in which the biosynthesis of 7α,25-OHC was blocked using the drug clotrimazole.

These intriguing studies illuminate a new role for oxysterols in the adaptive immune response. Continued characterization of the biological function of oxysterols will deepen our understanding the adaptive immune response and guide efforts in the design of new drugs for immune disorders. **Eva J. Gordon, Ph.D.**

Making Molecular Scaffolding

The molecular engineering of proteins and nucleic acids has enabled the creation of biomolecules with unique and varied applications, such as enhanced enzymatic activities, altered binding properties, and designed architectures. The three-dimensional structure and spatial organization of biomolecules can be an important component of their function, and development of new methods to fabricate unique molecular assemblies are at the center of diverse research efforts. Defined nanoscale architectures have been created by exploiting the inherent base-pairing properties of DNA, but such structures are generally created in an *in vitro* setting. Delebecque *et al.* (*Science* 2011, *333*, 470–474) now present the design and generation in bacteria of RNA assemblies that function as efficient scaffolding for an enzymatic reaction.



From Delebecque, B. G., *et al.*, *Science*, 2011, *333*, 470. Reprinted with permission from AAAS.

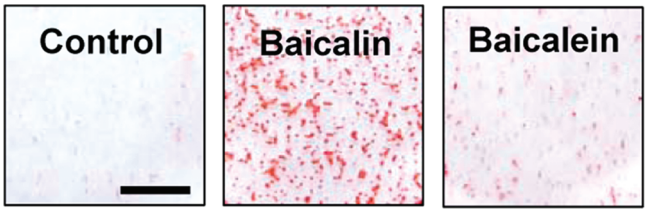
Use of RNA instead of DNA allows for exploitation of the transcriptional machinery in cells to produce RNA whose sequence can be designed to enable the efficient assembly into defined structures with specific binding capabilities. Discrete, one-dimensional (1D) and two-dimensional (2D) RNA assemblies were generated and characterized *in vitro* and in bacterial cells. Atomic force microscopy of various assemblies generated either via *in vitro* transcription or purified from cells demonstrated that the 1D assembly formed 1D RNA fibers suggestive of 1D ribbons, while the 2D assembly formed extended RNA fibers suggestive of RNA nanotubes. Transmission electron microscopy was used to examine the assemblies in whole bacterial cells, revealing that the 1D assembly formed thin filaments, while the 2D assembly formed compact spherelike structures. Finally,

Published: September 16, 2011

fluorescence complementation assays demonstrated that proteins assembled and interacted on the RNA scaffolds. To illustrate the utility of such RNA scaffolds, new assemblies were designed to bind two enzymes involved in the production of hydrogen such that a spatially constrained, enzymatically active structure was generated. Using gas chromatography, it was shown that hydrogen biosynthesis was increased in these cells. This study demonstrates the feasibility of engineering biological pathways within an intracellular environment using RNA-based scaffolds. **Eva J. Gordon, Ph.D.**

Baicalin and Bones

Maintaining bone strength and integrity is a continual balancing act, with bone removing cells called osteoclasts collaborating with their bone forming counterparts, osteoblasts. Osteoporosis, which literally means "porous bones", is a disease in which bones become thinner and less dense and fracture more easily, a process that can be attributed to either overachieving osteoclasts, sluggish osteoblasts, or a combination thereof. Commonly seen in postmenopausal women, osteoporosis has been linked to estrogen deficiency, but efforts to treat the condition with estrogen supplementation can lead to other serious conditions such as breast cancer and heart disease. Flavonoids, naturally occurring compounds found in many plants, have estrogen-like activities and are capable of affecting bone metabolism, but their mechanism of action is not well-defined. Guo *et al.* (*J. Biol. Chem.* 2011, *286*, 27882−27893) now use a cell-based screen to explore the bone-promoting potential of flavonoids and uncover an unexpected mechanism by which a flavone called baicalin promotes osteoblast activity.

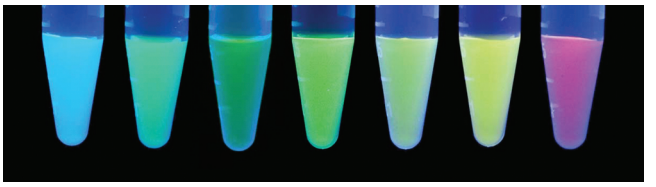


Guo, A. J. Y., *et al.*, *J. Biol. Chem.*, 2011, *286*, 27882−27893. Copyright 2011 The American Society for Biochemistry and Molecular Biology

The known ability of flavonoids to affect bone metabolism prompted the authors to conduct a screen of flavonoids from different subclasses, including flavanones, flavones, and isoflavones, for their ability to induce the differentiation of immature osteoblasts. The flavone baicalin, though notably not a related, deglycosylated flavone called baicalein, was found to induce the expression of numerous proteins associated with osteoblastic differentiation and to promote cultured osteoblasts to undergo a bone mineralization process. Notably, the osteoblast-promoting activity of baicalin was demonstrated to proceed not through interaction with estrogen receptors, but by activation of the Wnt/β-catenin signaling pathway, which is known to play an important role in the induction of bone formation. This mechanism of action points to the potential of baicalin and related flavonoids as improved therapeutic agents or food supplements for the prevention or treatment of osteoporosis, as they may not cause the known harmful effects that can result from activation of the estrogen receptor. **Eva J. Gordon, Ph.D.**

RNA Goes Green

At the heart of GFP is a vibrant fluorophore formed through the autocatalytic reaction of three amino acid side chains. This 4-hydroxybenzlidene imidazolinone, or HBI for short, is not fluorescent on its own, relying upon the protein environment that encapsulates it to generate the green glow upon excitation. Minor changes to the protein lead to new fluorescence properties, resulting in the rainbow of reporter colors used by cell biologists today. Now, a radical new approach by Paige *et al.* (*Science* 2011 333: 642−6) to generating HBI fluorescence takes the protein entirely out of the picture, only to replace it with RNA instead.

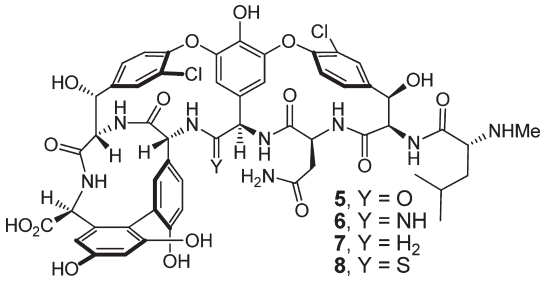


From Paige, J. S., *et al.*, *Science*, 2011, *333*, 642. Reprinted with permission from AAAS.

The authors used an *in vitro* evolution protocol to select RNA aptamers that bound to the dimethoxy derivative of the natural GFP fluorophone, DMHBI. After 10 rounds, one aptamer of approximately 60 nucleotides, termed 13-2, exhibited the highest green fluorescence upon binding to DMHBI. Numerous other aptamers showed fluorescent properties with DMHBI and these RNAs could bind with various HBI derivatives to generate interesting spectral differences. Upon optimization, difluoro-HBI (DFHBI) and an aptamer termed 24-2 were chosen for further characterization since this complex, dubbed Spinach, displayed a remarkable brightness over half that of EGFP protein. To move from the test tube to the Petri dish, the aptamer was then used to tag RNAs within living cells. The 24-2 aptamer was fused to the 3′ end of a small rRNA, 5S, and introduced into human kidney tissue culture cells. Upon treatment with DFHBI, the cells showed the pattern expected of 5S rRNA and even tracked with endogenous 5S to P granules in the cytoplasm during a sucrose stress test. Importantly, the DFHBI did not show fluorescence alone and did not promote cell death. Spinach fluorescence appears soon after transcription and is not as sensitive to photodamage as the fluorescent proteins. With this new fluorescent reporter strategy, many new cell biology experiments can be envisioned whereby a cellular RNA is tracked directly rather than indirectly through binding to a fluorescent protein or oligonucleotide. **Jason G. Underwood, Ph.D**

Overcoming Vancomycin Resistance

MRSA infections are caused by highly virulent and difficult to treat methicillin-resistant *Staphylococcus aureus*. These strains are resistant to commonly prescribed antibiotics against *Staphylococcal* infections, such as β-lactams. Although typically considered a nosocomial infection, recently MRSA infections have also originated from community sources. In the fight against MRSA, vancomycin emerged as a well-established, effective antibiotic of last resort. A recent and troubling rise in vancomycin-resistant MRSA strains has alarmed health care providers. To address this significant problem, Xie *et al.* (*J. Am. Chem. Soc.* 2011, DOI: 10.1021/ja207142h) report the development of a vancomycin analogue with potent antimicrobial activity against vancomycin resistant bacteria.

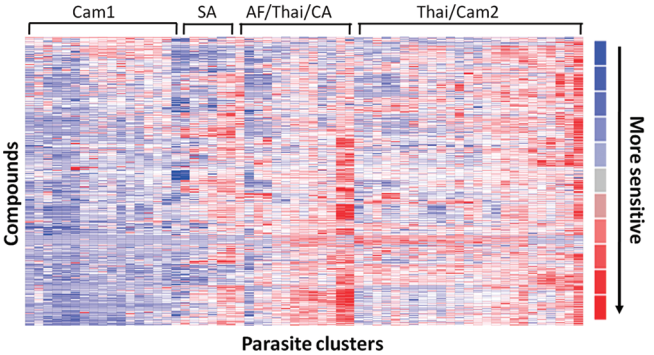


Reprinted with permission from Xie, J., *et al., J. Am. Chem. Soc.*, DOI: 10.1021/ja207142h. Copyright 2011 American Chemical Society.

The mode of action of vancomycin is via inhibition of cell wall biosynthesis. This antibiotic binds to D-Ala-D-Ala peptides of peptidoglycan and prevents essential cell wall cross-linking. However, vancomycin-resistant bacteria have found a way to thwart the antimicrobial activity of this antibiotic by modifying the D-Ala-D-Ala precursor peptide to D-Ala-D-Lac, resulting in a 1000-fold decrease in vancomycin potency. The change from a D-Ala-D-Ala to a D-Ala-D-Lac is due to the underlying substitution of an amide to an ester (NH→O). The authors used this observation to design a new vancomycin analogue that targets both D-Ala-D-Ala and D-Ala-D-Lac precursor peptide. Specifically, a [Ψ[C(NH)NH]Tpg4]vancomycin aglycon was developed that had a complementary (O→NH) modification in the vancomycin structure to mitigate the effects of the substitution in the precursor peptide. The new vancomycin analogue exhibited strong binding to both D-Ala-D-Ala and D-Ala-D-Lac peptides and showed potent antimicrobial activity against vancomycin-resistant bacteria. The development of this new antibiotic via an exquisite rational approach to counter the threat of vancomycin-resistant bacteria provides a new paradigm for future development of antibiotics. **Jitesh A. Soares, Ph.D.**

Profiling malaria's drug response

Infectious disease pathogens have the insidious talent of evolving resistance to drug treatments. Researchers can find new drugs to treat the disease, but the risk of developing new resistance remains. Treating such diseases with a combination of drugs from the start can slow or even prevent the development of drug resistance. In a clever application of high throughput screening and large scale genetic assays, Yuan *et al.* (*Science*, 2011, *333*, 724−729) have now mapped the relationships between active antimalarial compounds and genetic patterns in various *Plasmodium* strains. The results describe how the use of drugs in particular regions has shaped the evolution of malarial parasites and provide a strategy for finding drug combinations that could keep resistance at bay.



From Yuan, J, *et al.*, *Science*, 2011, *333*, 724. Reprinted with permission from AAAS.

Initially the researchers screened a library of nearly 3000 known compounds for activity against 61 different lines of the *Plasmodium* parasite. Thirty-two of those compounds showed high activity against most of the strains, and 10 of those compounds did not have previously reported antimalarial activity. When they paired off compounds to look for correlated responses to particular parasites, Yuan *et al.* found groups of drugs whose responses correlated with that of established antimalarials such as artemisinin and mefloquine, which suggested that their activity and resistance mechanisms might be similar.

These analyses are also helping researchers better understand the genetic underpinnings of the development of drug resistance in malaria. The drug response of parasite strains to a subset of active malarial drugs clustered parasite strains based on their geographic origin of these strains. Genome-wide association studies and linkage analysis of recombinant parasites with specific genetic traits traced the drug response to just three different genetic loci: *prdhfr*, *pfmdr1*, or *pfcrt*.

Chloroquine resistance is becoming increasingly common among malarial strains. The researchers used their genetic analyses and drug response profiles to look for drugs whose activity was different from that of chloroquine for possible pairings as combination treatments. These combinations were active, even against cloroquine resistant strains. In addition to providing a strategy for developing new antimalarial combination therapies, this study provides ways to more clearly understand the mechanisms of drug resistance. **Sarah A Webb, Ph.D.**