Spotlight

RNA Goes Mobile

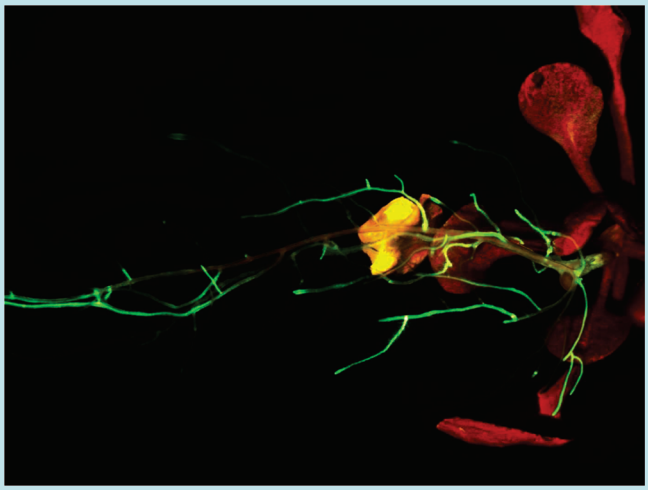


Image supplied by author.

In less than 20 years, the topic of "post-transcriptional gene silencing" in plants has transformed into the lively field now known as RNA interference or RNAi. Today, with more genomic and expression data in hand, researchers have uncovered the hallmarks of the RNAi or miRNA pathways, including small RNAs and particular protein players, among widely divergent eukaryotes. The silent organisms where studying silencing all began, the plants, employ diverse mechanisms of using small RNAs to regulate cellular functions and fight off viruses. In addition, RNAi spreads through the vasculature of the plant contributing to both cell−cell communication during development and the immune response after a viral attack. Now, two new studies find that the identity of the traveling signal is actually the small RNA duplexes themselves. Impressively, the two groups came to several of similar complementary conclusions, but using rather different approaches to the same question.

To study the phenomenon of RNAi spreading in plants, the study by Molnar *et al.* (*Science* 2010, *328*, 872−875) combined plant grafting techniques with a useful battery of transgenic *Arabidopsis* genotypes. Starting with a plant genotype expressing a hairpin construct to silence GFP, roots from various genotypes were then grafted on as a test. When roots that express GFP were fused to the shoots of the hairpin-expressing plant, the GFP was efficiently silenced. Then, the researchers turned to mutants for the Dicer-like (DCL) proteins, the enzymes necessary for generating small RNAs from longer double-stranded RNAs. With this arrangement, the small RNAs found in the DCL mutant roots must be from biogenesis and migration from the plant's shoots through the graft. High-throughput sequencing generated a complete small RNA profile for the roots and showed that thousands of GFP-targeting and endogenous small RNAs are mobile. Then, a fortuitous clue to the function of mobile 24 nucleotide RNAs arose from the enrichment of small RNA data at genomic locations harboring transposons or methylated DNA. The group tested the methylation status of several loci in the root cells. If the plant was not competent for 24 nucleotide small RNA production, the loci did not display proper methylation. If the grafting conditions fused a shoot that can make small RNAs to roots that cannot, the methylation in the roots was rescued, indicating that mobile small RNAs can reach the roots and direct methylation.

While the Molnar *et al.* study noticed an enrichment of mobile 23−24 nucleotide RNAs, a parallel study zeroed in on mobile silencing by the products of DCL4, a Dicer-like protein that produces 21 nucleotide RNAs. Dunoyer *et al.* (*Science* 2010, *328*, 912−916) used the SUC:SUL reporter genotype where silencing can be visualized by leaf coloration. With this strain, mobile silencing was readily visible since plant tissue 10−15 cells away from the vasculature lost its green coloration and displayed white veins. This patterning is induced by a silencing trangene against the SUL gene expressed only in a phloem-companion cell promoter specific manner. Another clever trick, the viral P19 protein, was expressed in the same cells as the silencer construct. The P19 protein binds specifically to small silencing RNAs and blocks the proper loading into effector complexes. When P19 was expressed in this manner, the white veins were now missing, indicating that the mobile silencer signal must be the now-sequestered 21 nucleotide RNAs. Additional tests supported the notion that the signal is the free duplex RNA rather than a form that has already bound and programmed the effector protein, AGO1. Finally, movement of small RNAs was tested directly using particle bombardment. When a GFP-expressing plant seedling was bombarded with particles coated in GFP-silencing small RNAs, a spread of the silencing signal was observed over the following days. Spread of the small RNA was also visualized directly by using chemically synthesized small RNA duplexes containing a terminal fluorophore.

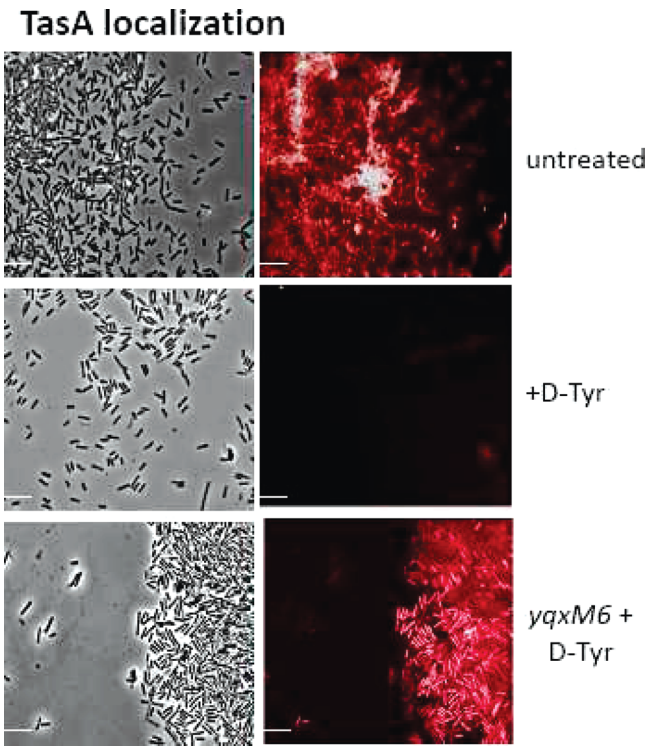
Together, these studies indicate that small RNAs are mobilized to neighboring cells and through the plant's vasculature to downregulate mRNA expression and guide DNA modification. The array of tools used by both groups indicates that there are many ways to study this interesting phenomenon and a multifaceted approach will be key to uncovering the next discoveries in how plant biology and viral defense are modulated by small RNAs on the move. **Jason G. Underwood, Ph.D.**

Published online June 18, 2010 • 10.1021/cb100150z

Spotlight

D-Amino Acids Bust Biofilms

Bacteria cluster into biofilms, communities linked by polysaccharides and protein that protect individual organisms from environment stresses. These transient groupings facilitate bacterial survival, but as nutrients are depleted and waste accumulates, single-celled organisms can be better off striking out on their own. This bacterial survival strategy has serious consequences in industry and medicine: biofilms foul water treatment and paper production plants and underlie many medical infections. A better understanding of the chemistry that controls these groupings could help scientists and medical professionals develop new sterilization techniques.



From Kolodkin-Gal, I. *et al.*, *Science*, 2010, *328*, 627. Reprinted with permission from AAAS.

Kolodkin-Gal *et al.* (*Science*, 2010, *328*, 627−629) have now described the mix of chemical signals from D-amino acids that trigger biofilm disassembly in *Bacillis subtilis* and other organisms. Because bacteria are known to produce D-amino acids, the researchers screened these compounds and found that the D-isomers of tyrosine, leucine, tryptophan, and methionine each blocked new biofilms. D-Tyrosine was the most potent of the individual amino acids (3 μM), but a cocktail of all four stops biofilm formation at concentrations as low as 10 nM. The researchers demonstrated with liquid chromatography and mass spectrometry that bacteria produce D-amino acids at sufficient concentrations to inhibit biofilm formation.

These D-amino acids incorporate into bacterial cell walls, interfering with the anchoring of protein fibers that bridge between bacteria in biofilms. In particular, bacteria treated with D-tyrosine show few of these anchoring fibers made from the protein TasA. Bacteria that are resistant to D-tyrosine's biofilm blocking effects show changes in the YqxM protein, which binds to TasA.

D-Tyrosine and the four D-amino acid cocktail also blocked biofilm formation in the pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results—and the ability of a wide range of bacteria to produce D-amino acids—suggest that these molecules might be widely useful as biofilm-busting agents.
Sarah A. Webb, Ph.D.

The LD Debate Revisited

In all kingdoms of life, information is stored and reactions are catalyzed by polymers synthesized from small building blocks. The polymerases that make RNA or DNA require nucleoside triphosphates as monomers, while proteins are put together from amino acids carried to the ribosome by tRNAs. At the heart of these processes are elegant biological catalysts, each requiring the proper stereochemistry for the reactions to proceed.

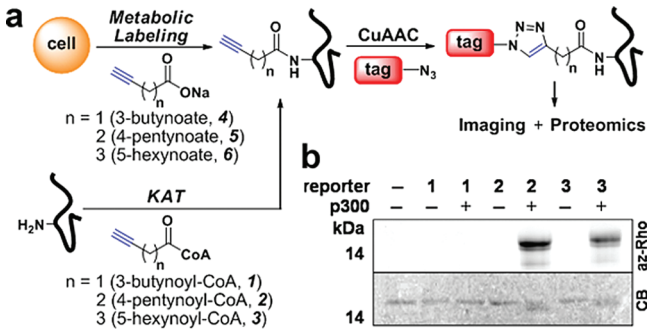
On Earth, all proteins are built from amino acids possessing the L-chirality, while sugars like those present in DNA, RNA, and cell's energy currency, ATP, all display D-chirality. A mystery ensues when one considers the first simple amino acids or sugars arising from a prebiotic soup. Without the influence of catalysts, one might expect the simple chemical reactions present in the soup to yield equal ratios of both enantiomers for any simple molecules. A clue to the L-amino acid mystery literally fell from space in the form of a meteorite from which a special class of methylated amino acids were discovered and appeared to induce a bias toward the L-chirality. An equal ratio of L + D amino acids results in a loss of solubility in water, so even a tiny increase of one enantiomer over the other could be amplified and account for the domination of L-amino acids in modern biology. But what could account for the domination of D-sugars on Earth?

A new study by Breslow and Cheng (*Proc. Natl. Acad. Sci. U.S.A.* 2010, *107*, 5723−5725) indicates that these two biases may have arisen in concert. The reaction of glycoaldehyde with formaldehyde should yield an equal ratio of L- or D-glyceraldehyde, a basic building block used to make biological sugars. Interestingly, the authors found that addition of any one of six L-amino acids to this reaction resulted in a bias toward the D-form. Addition of water to the situation allowed the amplification to occur by selective precipitation of a D + L glyceraldehyde complex. The authors also point out that a similar loss of solubility comes from D + L ribose nucleosides in equal ratio, so these RNA building blocks may have ended up with D-chirality due to an early bias and amplification. While this study

peers back in time with simple chemical concoctions, this possible connection between L-amino acids and D-sugars makes it an intriguing subject for further investigations. **Jason G. Underwood, Ph.D.**

Assessing the Aceytlome

Protein post-translational modifications, such as acetylation or phosphorylation, play pivotal roles in both normal and pathological processes. Protein acetylation, for example, is involved in the regulation of gene expression, and its misregulation has been implicated in numerous diseases. Characterization of the acetylome, the proteome of acetylated proteins, will facilitate the identification of acetylated proteins, as well as further elucidate the functional roles of protein acetylation. To this end, Yang *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 3640−3641) report the development of chemical tools for exploring the acetylome.



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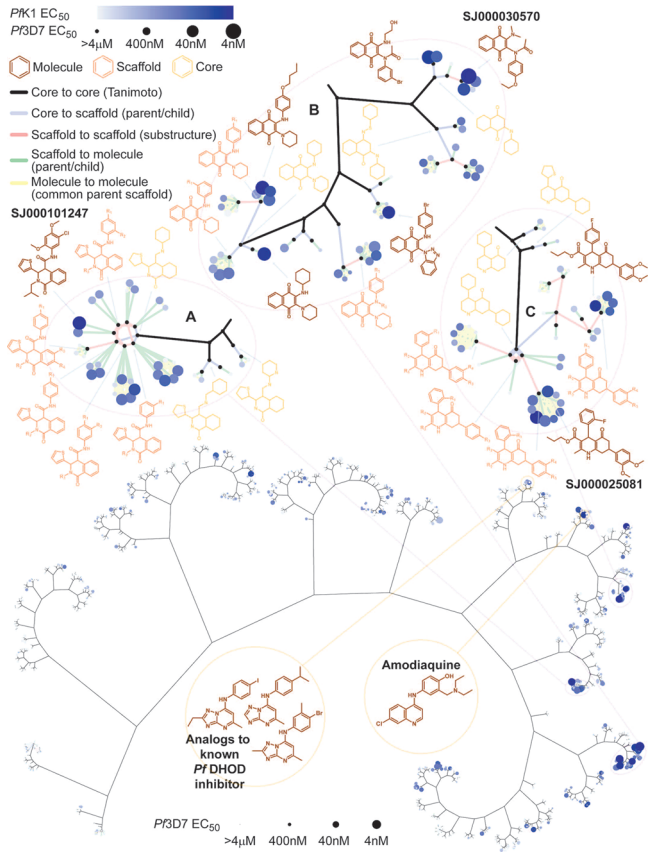
Exploiting the copper(I)-catalyzed azide−alkyne cycloaddition reaction as a bioorthogonal reaction for tagging acetylated proteins, alkyne-containing acetyl-CoA analogs were prepared. Exposure to a lysine acetyltransferase in the presence of a lysine-containing peptide substrate led to the generation of an acylated peptide bearing an alkyne group. Subsequent reaction of the peptide with an azide-containing fluorophore enabled detection of the peptide, as well as kinetic analysis of the reaction, revealing it to be efficient and robust. To investigate protein acetylation in live cells, alkynyl-acetate analogs suitable for metabolic incorporation into proteins were added to cells, and subsequent exposure to the fluorescent azide revealed that many proteins in the cell were acetylated. Addition of a biotinylated azide in place of the fluorescent azide enabled purification and identification of the acetylated proteins, and mass spectrometry analysis confirmed the lysine-acetylation of many known proteins as well as a number of new candidate proteins. Notably, distinct acetylome signatures were identified in different cell lines, pointing to the functional diversity of acetylation in different cell types as well as the utility of these analogs as general protein acetylation probes. These tools point to the design of similar agents for the examination of related enzymes, paving the way toward a more detailed understanding of the function and regulation of protein acetylation. **Eva J. Gordon, Ph.D.**

Leading the Way To Malarial Leads

According to alarming statistics, nearly 1 million people died from malaria in 2008, and close to 250 million people worldwide are afflicted with the disease. Malaria is caused by parasites of the *Plasmodium* species, which target and kill red blood cells upon transmission to humans through the bite of an infected mosquito. The emergence of drug-resistant *Plasmodium* strains has compromised the efficacy of clinically used antimalarial drugs, and few new chemical classes that effectively target the disease have been reported. Now, two studies (Gamo *et al.* (*Nature* 2010, *465*, 305−310) and Guiguemde *et al.* (*Nature* 2010, *465*, 311−315)) reveal the identification of thousands of compounds with antimalarial activity, with the hope that they will find use as drug leads for the malarial research community.



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In the study by Gamo *et al.*, nearly 2 million compounds were screened for their ability to inhibit the growth of a laboratory strain

as well as a drug-resistant strain of *Plasmodium falciparum* in human red blood cell cultures. Two methods were employed to structurally characterize the 13,533 inhibitors identified in the screens: molecular frameworks and fingerprint clusters. The molecular frameworks approach describes the core template of the compounds, whereas the fingerprint clusters method focuses on more subtle substituent patterns. Together, the characterizations enabled classification of the inhibitors into distinct groups, which facilitates the deciphering of their potential mechanisms of action. Analysis of the known biological activity of the compounds previously tested in other assays revealed over 400 human targets, including numerous G-protein coupled receptors and protein kinases, as well as several microbial proteins, as starting points for target identification. Indeed, comparison of the targets with orthologs in *P. falciparum* pointed to over 50 candidate drug targets in the parasite. Notably, the malarial kinome represents an exciting and relatively untapped source of potential drug targets for malaria. Moreover, structural comparison of the tertiary structures of numerous other potential targets, including GPCRs, nuclear receptors, ion channels, and transporters, that lack primary sequence homology to proteins in *P. falciparum* may also offer clues to the identity of new malarial target proteins. Finally, it is possible that some of the inhibitors targeted a specific interaction between the parasite and human red blood cells, hinting at yet another potential drug discovery strategy for this important and devastating disease.

In a related study, Guiguemde *et al.* screened over 300,000 molecules for their ability to inhibit growth of *P. falciparum* in red blood cells. From the initial identification of ~1300 hits, compound validation and structural analysis led to the selection of 172 compounds for further characterization. Using enzyme inhibition and thermal melt shift assays, the compounds were tested against 66 potential malarial targets, including the high priority targets dihydroorotate dehydrogenase, hemozoin formation, and falcipain-2. Notably, 19 compounds inhibited one of these three targets, validating this approach for the identification of potential antimalarial drugs. Furthermore, 15 compounds were shown to bind to 7 distinct malarial proteins, unveiling potential new malarial targets. The compounds were characterized further by assessing their efficacy against drug-resistant strains, examining their selectivity for *P. falciparum* compared with three other parasite species, and evaluating their pharmacokinetic properties. On the basis of these characterizations, one compound was selected for testing in a mouse model of malaria. Notably, treatment with the compound resulted in 90% suppression of parasite levels in the blood.

The prevalence and persistence of malaria throughout the world speaks to the urgent need for effective new treatments for this devastating disease. Screening efforts such as those described here provide desperately needed new chemical classes as jumping off points for development of new antimalarial drugs, as well as offering clues to the identity of untapped malarial drug targets. **Eva J. Gordon, Ph.D.**