Eliminating Malaria

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each H_2 unit lost; those electrons can be used to activate the N_2 unit.

Shima *et al*. show how the trimetallic heptahydride $Cp'_3Ti_3H_7$ (see the figure, **1**) can activate and cleave one equivalent of N_2 under very mild conditions to form a complex with one nitride bound to the three titanium atoms at the center and one bridging imide (the Ti(NH)Ti unit, see the figure, **2**), accompanied by the release of two equivalents of dihydrogen (H_2). Further reaction with N_2 under more forcing conditions (180°C, no solvent) results in a triimido-nitride species (see the figure, **3**). The authors provide compelling experimental evidence on exactly how these reactions proceed and back up their experiments with computational results.

What is particularly appealing about this report is the data on proposed intermediates during the transformation that generates compound **2**. Monitoring the reaction of $^{15}N_2$ with the heptahydride **1** at low temperatures shows evidence for the formation of two intermediates. The first intermediate is an end-on side-on bound N_2 complex formed through loss of H_2. This is followed by scission of the N-N bond to generate a di-μ-nitride trihydride. The latter species slowly transforms to the imide-nitride **2** via a reductive elimination of one of the bridging hydrides to a nitride to generate a N-H bond. Both transformation processes are unprecedented in the dinitrogen activation literature. The N-H–forming process mimics one of the steps suggested on iron surfaces for the formation of ammonia in the Haber-Bosch process, albeit with different metals.

The biological process of nitrogen fixation involves the enzyme nitrogenase and a cofactor containing a multi-iron site that binds N_2 and converts it to ammonia. The detailed mechanism of ammonia formation from atmospheric N_2 by nitrogenase remains to be resolved, but it has recently been suggested (*7*) that the initial N_2 binding step involves H_2 elimination from the cofactor, perhaps from iron hydride moieties at the active site. The trititanium system reported by Shima *et al*. is by no means a model for nitrogenase, but both systems involve multimetallic sites, which may be beneficial for N_2 reduction.

Clearly, having more than two metal centers and multiple hydrides present allows more N_2 to be activated, along with the formation of N-H bonds. But it is not as simple as more-is-better. Shima *et al*. report that tetrametallic hydride clusters of the formula $Cp'_4M_4H_8$, where M is Ti, Zr, and Hf, do not react with dinitrogen under any circumstances. Future studies should investigate how other multimetallic sites may be used to activate small molecules, especially nature's most inert diatomic molecule, N_2.

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MICROBIOLOGY

Eliminating Malaria

Global eradication requires concerted efforts to combat emerging resistance to the potent antimalarial artemisinin.

David A. Fidock

Malaria kills more young children than any other infectious disease. The most pernicious causal agent, the protozoan parasite *Plasmodium falciparum*, is responsible for the death each year of more than half a million children, mostly in sub-Saharan Africa. Until recently, control efforts were thwarted by pyrimethamine- and chloroquine-resistant parasites, whose appearance in Africa was traced back to origins near the Thai-Cambodian border. Fortunately, the discovery of the potent antimalarial properties of artemisinin (*1*) has helped turn the tide against malaria. Artemisinin-based combination therapies (ACTs), which combine a potent but short-lived artemisinin derivative with a longer-lasting partner drug, have now been officially adopted across virtually the entire malaria-endemic world. Their deployment, along with efforts to distribute insecticide-treated bednets, is associated with recent substantial reductions in malaria burden. However, recent studies from Cambodia and now Thailand show that once again resistance is looming as a major threat to global control efforts (see the figure) (*2*, *3*).

Emerging resistance to artemisinins is defined as a reduced rate of parasite clearance after administration of an artemisinin derivative such as artesunate or an ACT. In western Cambodia, the epicenter of resistance, artesunate treatment yielded a mean clearance half-life of 5.9 hours in Pursat, as opposed to 2 hours for a comparator drug-sensitive cohort in Wang Pha, western Thailand (*4*). Delayed clearance translates into an increased proportion of individuals having microscopically detectable blood-stage infections by the third day of treatment and raises the risk of parasite recrudescence (i.e., disease reappearance). Major efforts are under way in this region to eliminate malaria while ACTs remain clinically effective, with the theory that nothing short of elimination will prevent its global dissemination.

Mode-of-action studies, while controversial, mostly converge on the idea that artemisinins can be activated in the parasite via iron-mediated scission of the peroxide bridge following hemoglobin proteolysis and release of ferric heme iron (*5*). This can lead to the formation of carbon-centered radicals that trigger cell death. This mechanism can account for drug action during most of the 48-hour intraerythrocytic developmental cycle, although how artemisinins act against the very early "ring" stages that form shortly after host cell invasion remains enigmatic.

In vitro studies on *P. falciparum* cultures exposed to high concentrations of artemisinin show that parasites can acquire an initial state of tolerance (*6*) whereby early ring-stage parasites adopt a drug-induced quiescent or dormant state and then resume normal growth upon drug removal (*7*). This trait is distinct from mechanisms of resistance to other antimalarials, which typically permit robust growth despite drug treatment and often involve genetic changes that alter drug targets or efflux systems (e.g., PfCRT or PfMDR1). Further research is needed to define the molecular basis of in vitro tolerance or resistance, understand its relation to delayed clearance rates, and model how resistance, parasite fitness, transmission intensity, and treatment coverage collectively influence the spread of resistance.

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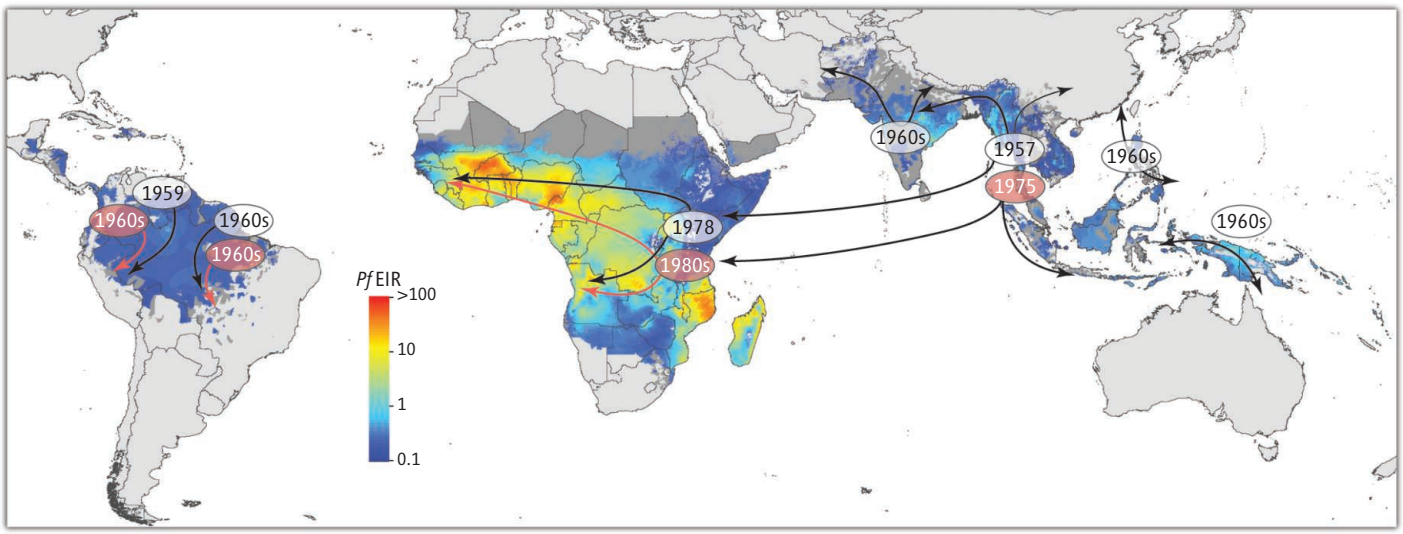

Spread of *P. falciparum* resistance. Appearance and spread of resistance to former first-line antimalarials chloroquine and pyrimethamine (clear and orange ovals, respectively), showing migration from Southeast Asia to Africa. Emerging resistance to artemisinins is now documented in Southeast Asia, raising concerns about its future migration to Africa. Resistance patterns are overlaid onto a geostatistics map of *P. falciparum* entomological inoculation rates (EIR; numbers of infectious mosquito bites per individual per year) modeled for 2010 (*16*).

Recent high-density genotyping studies with clinically defined Southeast Asian patient isolates showed that parasite clearance rates have a predominantly heritable component, which was associated with a 35-kb region on chromosome 13 (*8*). A separate genome-wide association study also associated delayed clearance with single-nucleotide polymorphisms on chromosomes 13 and 10 (*9*). The chromosome 13 candidate markers identified in these two studies did not overlap, suggesting intrinsic difficulties in obtaining sufficiently high resolution to identify the causal gene and/or the presence of multiple genetic determinants on that chromosome.

Novel insights into the genetic basis of artemisinin resistance were recently provided by whole-genome sequencing of 825 parasite strains from Asia and Africa, which identified a highly unusual population structure in parasites from western Cambodia (*10*). This region harbors four genetically highly differentiated but nonetheless sympatric parasite subpopulations termed KH1 to KH4. Three (KH2, 3, and 4) showed a significant prolongation of parasite clearance half-life relative to the KH1 subpopulation that was commonly observed in largely artemisinin-sensitive neighboring regions. One explanation might be the presence of multiple genetic loci that collectively confer upon each subpopulation the ability to survive artemisinin exposure; independent segregation of these genes during sexual recombination would thus be selected against during treatment. Alternatively, the lack of recombination may reflect relatively recent founder events that separately acquired a primary resistance determinant and that have not yet had time to recombine frequently and disrupt linkage disequilibrium. In KH2 parasites, chromosome 13 showed extensive linkage disequilibrium, with a single haplotype extending across half the 4-Mb chromosome; this makes the identification of specific changes associated with delayed clearance rates particularly challenging. Among the genome-wide candidates were several transporter genes that have highly differentiated sequences in the founder subpopulations, including the ABC transporters PfMDR1 and PF13_0271 that might restrict drug access to its site of action.

This study also identified substantial changes in the DNA mismatch repair pathway, including the repair heterodimer MutLα (consisting of PMS1 and MLH1) and its physical partner UvrD (*10*). Resistance-associated mutations in DNA repair pathways were separately observed for Rad5 (*9*), which is involved in the DNA damage tolerance pathway of postreplication repair (*11*). Mutations in this pathway have been implicated in cell cycle arrest in yeast and might play a similar role in *P. falciparum*. The presence of DNA repair variants in the KH1 subpopulation also suggests that some changes may predate artemisinin resistance—for example, by creating initial hypermutator strains that accelerate the frequency of resistance. This finding recalls the earlier report of an ARMD (accelerated resistance to multiple drugs) phenotype in some Southeast Asian parasites (*12*). Interestingly, some MLH1 variants in yeast can suppress meiotic crossovers (*11*), raising the possibility that the changes in *P. falciparum* might be used to preserve linkage disequilibrium and polygenetic traits.

Molecular tools are urgently needed to monitor artemisinin resistance and to identify therapeutic approaches to contain it. Forward genetic methods, which entail crossing drug-resistant and drug-sensitive parasites and which previously localized the genetic determinants mediating resistance to chloroquine and pyrimethamine, would be useful if the trait followed a pattern of Mendelian inheritance. This trait could be quantified using an in vitro assay recently used to study parasite ring-stage susceptibility to artemisinins (*7*). Such crosses, which until now have required nonhuman primates, may be achievable using a new human hepatocyte- and erythrocyte-engrafted FRG NOD mouse model that permits mosquito-delivered *P. falciparum* parasites to progress through the liver and be recovered as blood-stage forms (*13*). Reverse genetic approaches, where candidate genes are modified via allelic exchange and phenotypic changes assessed, also benefit from the recent development of customized zinc finger nuclease–mediated gene editing in *P. falciparum* (*14*). This makes it feasible to assess a panel of candidates, with the goal of defining DNA sequence markers to inexpensively and rapidly screen for the spread of resistance, and begin to delineate its molecular basis. Finally, taking inspiration from approaches in *Mycobacterium tuberculosis* (*15*), new high-throughput screening approaches are required to identify antimalarial compounds that effectively eliminate artemisinin-tolerant quiescent parasites.

Emerging resistance to artemisinins has not yet compromised the outstanding

clinical efficacy of ACTs across most of the malaria-endemic regions of the world; even in western Cambodia most infections are reported to clear after ACT treatment. Malaria elimination remains an achievable goal, one that is critically dependent on an expanded and unified vision coordinated among funders, governments, health care providers, scientists, and the pharmaceutical industry. The issue of emerging artemisinin resistance highlights the necessity to intervene at all levels to prevent a looming disaster, and an opportunity to bring about a truly global accomplishment that directly or indirectly benefits us all.

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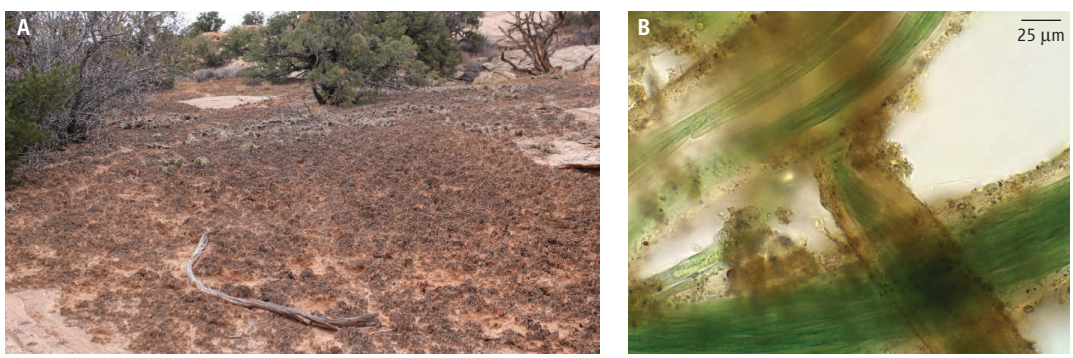
MICROBIOLOGY

Some Like It Hot, Some Not

The microorganism composition of dryland soils depends on regional climate.

Jayne Belnap

Dryland ecosystems cover over 40% of Earth's terrestrial landmass (*1*). Biocrusts—soil communities consisting of cyanobacteria, mosses, and lichens—can cover up to 70% of the ground in these ecosystems (see the figure, panel A) (*2*). The crucial role played by these and other very small organisms in nutrient, carbon, and water cycles has become increasingly clear in the past few decades (*2*, *3*). Soil stability and the composition and performance of vascular plant communities also depend on biocrust health and activity. Yet, little is known about the identity, biology, ecophysiology, or distribution of the microbial components that dominate biocrusts (*4*, *5*). Data are also needed to understand how they will respond to climate change. On page 1574 of this issue, Garcia-Pichel *et al*. (*6*) take a first step in filling this data gap.



Hidden variation. (**A**) Soils covered by biological soil crusts near Moab, Utah. Because of low plant cover in drylands, biological soil crusts can comprise 70% or more of total cover and mediate most inputs and outputs from the soils. (**B**) *Microcoleus vaginatus*. Garcia-Pichel *et al.* show that biocrusts are dominated by *M. vaginatus* in colder deserts and *M. steenstrupii* in hotter deserts. As global temperatures increase, it is likely that *M. steenstrupii* will replace *M. vaginatus* in many deserts. Although the two species are morphologically similar, the ecological implication of this replacement is unknown. Movie S1 shows *M. vaginatus* filaments moving within a common polysaccharide sheath.

Using samples from western U.S. desert sites across a range of climatic regimes, the authors compared DNA signatures of known cultivated cyanobacterial isolates to those in their field samples. They found a clear pattern of biogeographic segregation: At most sites, one of two cyanobacterial species dominated, with *Microcoleus vaginatus* (see the figure, panel B, and movie S1) prevalent at colder sites and *M. steenstrupii* at warmer sites. The authors then conducted physiological studies to confirm that growth of *M. vaginatus* was, indeed, favored at lower temperatures and *M. steenstrupii* at higher temperatures. They predict that the latter will likely replace the former as temperatures increase in the future.

The study illustrates the need to know what microorganisms are present at a site and how they affect ecosystem function, rather than exclusively focusing on the more visible macroorganisms such as plants, animals, or even mosses and lichens (*2*, *4*, *7*). Modern genetic studies of microbes can quickly and cheaply produce overwhelming amounts of data, but adding the functional dimension to molecular diversity has been exceedingly difficult. There is an urgent need for usable, consistent, and agreed-upon taxonomic and functional categories for microbes.

As shown by Garcia-Pichel *et al*., ecologically meaningful insights into microorganisms can be obtained by coupling new techniques (such as high-throughput molecular surveys of community DNA) with traditional techniques such as cultivation (*7*) and ecophysiological characterization of relevant isolates. (This approach, of course, is limited to organisms that can be cultured.) As it turns out, *M. steenstrupii* (and the nitrogen-fixing cyanobacterium *Scytonema*) appear to be far more important in hotter deserts than previously recognized.

Understanding the large difference in microbial composition in biocrusts from different regions will be crucial for managing these communities under future conditions. At colder sites, a shift from the dominant *M. vaginatus* to *M. steenstrupii* with warming could have a large effect on the ecosystem services provided by biocrusts. The extent of

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