PERSPECTIVES: BIOMEDICINE

Planting the Seeds of New Antimalarial Drugs

Robert G. Ridley

The *Plasmodium* parasite that causes malaria is a member of a group of one-celled organisms called apicomplexans. The malaria parasite and other apicomplexans have an unusual, self-replicating organelle, the apicoplast, that has its own maternally inherited (35 kb, circular) DNA, reminiscent of the separate DNA of mitochondria and chloroplasts. Roos has demonstrated that the replication of the apicoplast is essential for malaria parasite survival (*1*). The apicoplast, of algal origin, is associated with enzymes that are found in plant and bacterial (but not animal) metabolic pathways. Some of these enzymes have considerable potential as targets for antimalarial drugs (*2*–*4*). The fact that the apicoplast DNA encodes prokaryotic-like proteins associated with transcription and translation (*5*) probably explains the susceptibility of the malaria parasite to antibiotics such as doxycycline and azithromycin, which interfere with bacterial protein synthesis. On page 1573 of this issue, Jomaa *et al.* (*6*) reveal the existence of another apicoplast metabolic pathway not found in animals: the mevalonate-independent pathway of isoprenoid synthesis (see the figure). They propose that enzymes in the nonmevalonate pathway could be valuable targets for antimalarial drug development and identify two compounds that block the pathway and cure malaria in a mouse model of the disease.

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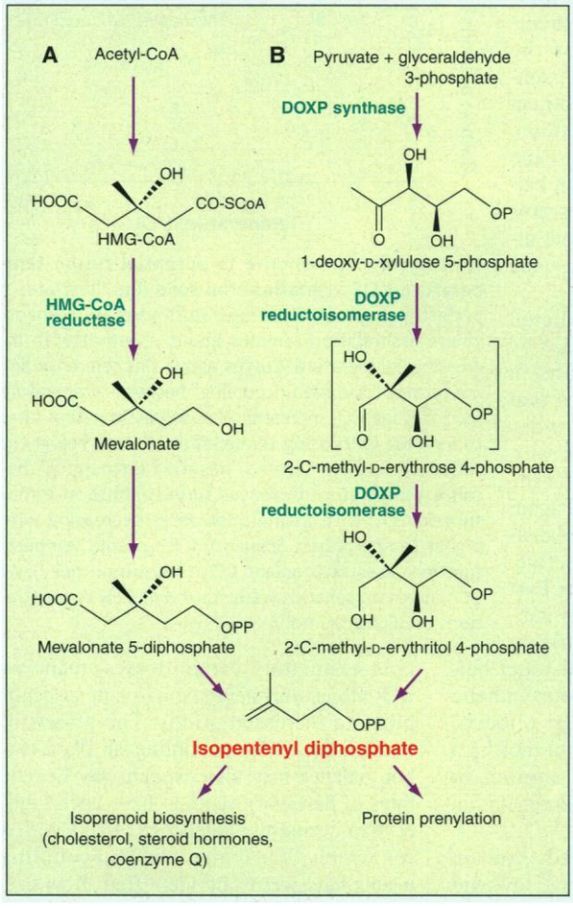
The essential subunit required for synthesis of isoprenoids (such as cholesterol and steroid hormones) is isopentenyl diphosphate (see the figure). In humans, other mammals and fungi, isopentenyl diphosphate is synthesized by the mevalonate pathway. In some eubacteria, algae, and higher plants, however, it is synthesized by the 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway (also called the methylerythritol phosphate pathway) that does not require mevalonate as an intermediate. Jomaa *et al.* rationalized that malaria parasites—which apparently lack HMG-CoA reductase (a key enzyme in the mevalonate pathway) but have an organelle associated with plant and bacterial metabolic enzymes—must synthesize isoprenoids by the DOXP pathway. From sequence data provided by the malaria genome project, they identified two genes in *Plasmodium falciparum* (the parasite causing the most lethal form of malaria) that encode key enzymes in the DOXP pathway—DOXP synthase and DOXP reductoisomerase. They showed by polymerase chain reaction that mRNA encoding these genes was present in the parasite as it grew within red blood cells and concluded that a functional DOXP pathway exists in malaria. They also elegantly demonstrated that an amino-terminal signal sequence in the *P. falciparum* DOXP reductoisomerase targeted the enzyme to apicoplasts when appropriate DNA constructs were transfected into an apicomplexan parasite related to malaria (*P. falciparum* is less amenable to such studies). This confirmed that the enzymes of the DOXP pathway are associated with the apicoplast.

Fortunately for the investigators, DOXP reductoisomerase had already been identified and evaluated as an antibacterial drug target by Fujisawa Pharmaceuticals. Two phosphonate compounds, fosmidomycin and FR900098, when given orally were found to inhibit the bacterial enzyme and the growth of certain bacterial species with minimal side effects. Jomaa and colleagues now demonstrate that these two compounds are able to inhibit growth of *P. falciparum* in culture and to cure mice infected with the related malaria parasite, *Plasmodium vinckei*. They conclude that the DOXP pathway is functionally active during growth of the malaria parasite inside red blood cells and that the pathway, and in particular DOXP reductoisomerase activity, is essential for parasite growth.

These encouraging results highlight several pressing questions. Is the complete DOXP pathway operational in malaria parasites and are the enzymes of the pathway all associated with the apicoplast? In higher plants, the DOXP pathway coexists with the mevalonate pathway, the DOXP pathway residing in the chloroplast and the mevalonate pathway in the cytoplasm. Is the DOXP pathway solely responsible for isopentenyl diphosphate biosynthesis in the malaria parasite and does the mevalonate pathway exist at all in this organism? Sole dependence on the DOXP pathway would probably raise the value of this pathway's enzymes as drug targets (although it should be noted that, despite the coexistence of both pathways in plants, fosmidomycin analogs still possess herbicidal activities) (*7*). The malaria genome sequencing project will be invaluable for addressing these questions.



Antimalarial DOXology. Synthesis of isopentenyl phosphate, the precursor of isoprenoids such as cholesterol, by the mevalonate (**A**) and DOXP (**B**) pathways. Animals have only the mevalonate pathway whereas green algae and some bacteria depend on the DOXP pathway. Both pathways exist in higher plants. In malaria parasites such as *P. falciparum*, the DOXP pathway is located in a special organelle, the apicoplast, and the mevalonate pathway is thought to be completely absent.

The author is with Drug Discovery Research and the New Medicines for Malaria Venture, Special Programme for Research and Training in Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

The authors mention that fosmidomycin and FR900098 had to be given three times a day owing to their short half-life in mouse plasma. A half-life of just a few hours in humans would mean multiple dosing over many days. This would significantly limit the use of these compounds as antimalarials in the clinic as it would be difficult to ensure compliance with such treatment regimens and so prevent reappearance of the parasites in blood (recrudescence). But, even if these two compounds do have a very short half-life in human plasma, they could conceivably be used in combination with other therapies. More significantly, given their low toxicity, these compounds could serve as valuable leads for the design of candidate antimalarial compounds with improved pharmacokinetic properties.

In the longer term, the validation of the DOXP pathway as a source of antimalarial drug targets opens up the possibility of screening large libraries of compounds for new chemical leads against DOXP reductoisomerase and other enzymes in the pathway. It is worth noting that antimalarial drugs that target a single enzyme, such as pyrimethamine (which inhibits dihydrofolate reductase) and atovaquone (which inhibits the mitochondrial cytochrome b/c1 complex) have ultimately found clinical application in synergistic drug combinations. Identifying inhibitors of several enzymes in the DOXP pathway might also result in synergistic drug combinations of increased therapeutic value.

Finally, it is worth acknowledging that sequence data from the malaria genome project have facilitated this work and will continue to be of benefit to future drug discovery efforts. As more data are generated from the malaria genome project, our understanding of the metabolic pathways in the malaria parasite will improve. As the genome sequence nears completion, one worthy goal is the generation of a complete *P. falciparum* metabolic map (metabolome) based on predicted gene products.

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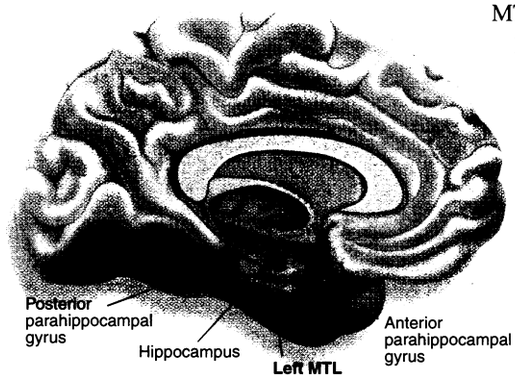
PERSPECTIVES: NEUROSCIENCE

Remembrance of Things Past

Daniel L. Schacter and Anthony D. Wagner

In a typical day, people experience myriad events and see innumerable objects, yet only some of these experiences are converted into enduring memories (*1*). Progress in understanding the neural pathways that encode these memories has been rather modest thus far. Typical studies of brain-injured amnesic patients (*2*) cannot cleanly distinguish between the effects of brain damage on the encoding of memories and their retrieval from storage (*3*). Although neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), detect activity in specific brain regions as people carry out various kinds of memory tests (*4*), their time resolution is not fine enough to determine the precise sequence in which different brain regions influence the encoding and formation of memories. Now, Fernandez *et al.* (*5*) track the serial encoding of memories within the medial temporal lobe (MTL) of the brain (a region beneath the temporal lobe surface that includes the parahippocampal gyrus and hippocampus) using a real-time electrophysiological technique (see the figure). They report on page 1582 an attempt to answer the fundamental question: Where and when are memories formed in the brain?

Previous studies (*6*) have used event-related fMRI (*7*) or electrophysiological techniques (*8*) to look at areas of brain activity during encoding of specific experiences that were subsequently forgotten or remembered. Study participants (*6*)—scanned by fMRI as they viewed a series of words and then tried to recognize them from a new list—showed increased brain activity during information encoding in the posterior region of the left MTL (also called the left parahippocampal gyrus) and in the left frontal lobe for words that were subsequently remembered compared to words that were subsequently forgotten. Comparable results were reported in subjects scanned as they studied pictures of everyday scenes, and later tried to remember them. But here, the increased fMRI signal during encoding for recalled pictures was located in both the left and right posterior MTL and in the right frontal lobe.



Memories are made of this. Lateral view of the brain highlighting three regions of the medial temporal lobe (MTL) that are involved in memory formation: the anterior parahippocampal gyrus (purple) and hippocampus (green) in the anterior MTL, and the posterior parahippocampal gyrus (red) in the posterior MTL.

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The fernandez study now shows that two parts of the left MTL—the anterior MTL in the rhinal cortex and the hippocampus proper—contribute to the memory encoding of words and their subsequent recall. The investigators conclude that the timing of the contributions of the two regions is staggered such that encoding activity in the hippocampus follows encoding activity in the anterior MTL. Fernandez *et al.* recorded electrical activity with long electrodes inserted into the MTL of 12 epilepsy patients in whom the MTL was unaffected. The event-related potentials (ERPs) measured by these depth electrodes provide fine-grained spatial resolution of brain activity (also available with fMRI) and real-time temporal resolution (which is not possible with fMRI). During electrical recording the patients were asked to memorize 12 words that were presented on a computer monitor. After a brief period of distraction, patients attempted to recall the words they had just read. In the anterior MTL, ERPs recorded for list words that were remembered versus those that were forgotten began to differ approximately 310 ms after stimulus presentation (that is, the negative potential was greater for remembered than for forgotten words). In the hippocampus, by contrast, ERPs for remembered and forgotten words did not begin to differ until approximately 500 ms after stimulus onset (in this case, there was a

The authors are in the Department of Psychology, Harvard University, Cambridge, MA 02138, and Massachusetts General Hospital Nuclear Magnetic Resonance Center, Harvard Medical School, Boston, MA 02129, USA. E-mail: dls@wjh.harvard.edu