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Spotlight

para-Aminobenzoate Synthesis versus Salvage in Malaria Parasites

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Enzymes of the folate *de novo* synthesis pathway in malaria parasites are proven antimalarial drug targets. A key precursor for folate synthesis is *para*-aminobenzoate (*p*ABA). In a recent study [1] (*Cell Rep.* 2019;26:356–363 e4), the contributions of *p*ABA synthesis versus salvage were re-evaluated in a rodent malaria model with knockout parasites grown in mice fed with various diets. The results imply that malaria parasites can either synthesize or salvage *p*ABA to meet the demand for folates.

Folates, derived from the Latin word *folium* (leaf), are abundant acids in green leaves and grain products, and are also concentrated in certain animal tissues (e.g., liver and kidney). Folates are central cofactors in one-carbon metabolism that are essential for synthesizing nucleic acids (thymidine and purines), methionine, and mitochondrial *N*-formyl-methionyl-tRNA [2,3]. Not only does folate cure megaloblastic anemia and prevent neural tube defects in fetuses, it also has beneficial effects in other health conditions [3]. While humans are folate auxotrophs, malaria parasites are able to synthesize it *de novo*. The folate biosynthesis pathway has been successfully exploited to yield a long list of antimalarial drugs targeting dihydropteroate synthase (DHPS) or dihydrofolate reductase-thymidylate synthase (DHFR-TS), two enzymes of this pathway [4]. For instance, for a long time sulfadoxine (a DHPS inhibitor) and pyrimethamine (a DHFR inhibitor) have been used in combination to prevent or treat falciparum malaria [5]. A key precursor for the synthesis of folate is *p*ABA (Figure 1), which could be derived either from *de novo* synthesis or salvage. Yet, the relative contributions of *p*ABA synthesis and salvage in malaria parasites have remained unsettled.

In 1952, Maegraith *et al.* observed that rats fed with milk were significantly less susceptible to *Plasmodium berghei* infections [6]. Shortly after, it was found that lack of *p*ABA was the main reason for poor parasite growth in animals on a milk diet [7]. Since then, it has been generally accepted that *p*ABA salvage from foods is the key to support parasite growth *in vivo*, and the putative *p*ABA *de novo* synthesis pathway from the precursor chorismate via two enzymes, aminodeoxychorismate synthase (ADCS) and aminodeoxychorismate lyase (ADCL), has been left uncharacterized. In the work just published by Matz *et al.* [1], the authors deleted ADCS and ADCL individually in *P. berghei* and observed that both knockout (KO) parasite lines were able to propagate normally in mice fed with a conventional fortified diet. For the first time, Matz *et al*. have provided clean genetic data showing that *p*ABA *de novo* synthesis is not essential for malaria parasites grown in animals with normal diets. In milk-fed mice, both ADCS and ADCL KO parasites suffered a severe growth arrest, which was abolished upon *p*ABA supplementation. Thus, *p*ABA salvage is sufficient to support parasite growth in mice fed normally, rendering *p*ABA *de novo* synthesis not essential under these conditions. One thing remaining to be clarified, however, is why KO parasites did not succumb completely in mice fed with milk alone, when both dietary *p*ABA and *de novo* synthesis were eliminated simultaneously. Although, with a lower parasitemia (sometimes undetectable), the ADCS KO parasites remained persistent in milk-fed mice for an extended time [1]. The authors reasoned that parasites might be able to salvage alternative precursors or folates to some degree. While milk contains a considerable concentration of 5-methyltetrahydrofolate (5-MTHF) [1], this compound is taken up very poorly by malaria parasites, but, in line with the authors' suggestion, *p*ABA monoglutamate, one of the degradation products of 5-MTHF, has been shown to be a reasonably good substrate for the

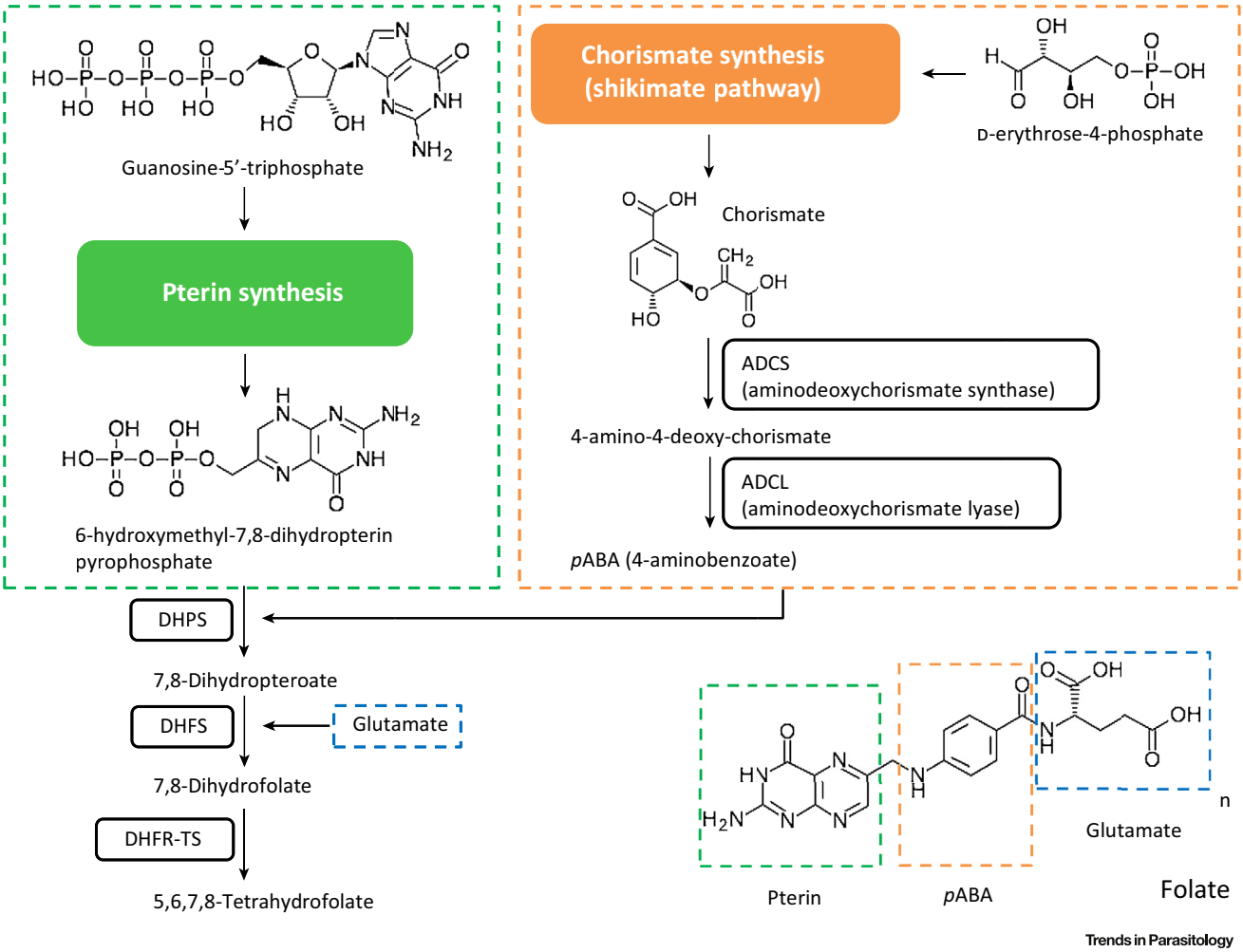

Figure 1. A Schematic Diagram of *p*ABA/folate *de novo* Synthesis. ADCS and ADCL synthesize *p*ABA from chorismate. Folates are generally mono- (circulating) or poly- (intracellular) glutamated. Abbreviations: ADCL, aminodeoxychorismate lyase; ADCS, aminodeoxychorismate synthase; DHPS, dihydropteroate synthase; DHFS, dihydrofolate synthase; DHFR-TS, dihydrofolate reductase-thymidylate synthase; *p*ABA, *p*-aminobenzoate.

Plasmodium falciparum folate transporters [8]. Additionally, Matz *et al*. observed normal growth of wild-type (WT) parasites in mice fed with milk alone (in the apparent absence of *p*ABA) [1], which suggests that the dominant view since the 1950s, that *p*ABA salvage plays a more critical role than *de novo* synthesis [9], requires modification. However, due to the complexity of animal husbandry, host microbiota (which can be a source of folates), and diet variations, whether *p*ABA *de novo* synthesis alone is absolutely sufficient to support parasite growth needs further verification. Since *P. falciparum* can be cultured *in vitro* using a defined medium, it might be a better model to tease out this issue.

In contrast to *p*ABA supplementation, which rescued ADCS or ADCL KO parasites to WT growth rates in milk-fed mice, supplementation with a normal concentration of folic acid (FA, 1.3 mg/kg) supported only a slow growth of KO parasites. The use of a higher concentration of FA (10 mg/kg), however, restored growth of KO parasites to near the WT rate [1]. This is consistent with historical data from the 1960s showing that FA could only partially substitute for *p*ABA [9].

While fast-growing blood-stage parasites require significant *p*ABA salvage or *p*ABA *de novo* synthesis for optimal growth, malaria parasites apparently have a much reduced requirement for folates in mosquito stages. From the time female and male gametes fuse in the mosquito gut, it takes about 10–14 days to form just a couple of thousand progeny from each zygote [10]. Thus, malaria parasites grow at their slowest rate during the mosquito

stages. Since folate metabolism would still be essential even under slow-growing conditions, malaria parasites must salvage enough folate-related compounds either from the blood meal or from the mosquito host. In either case, a lower demand for *p*ABA/folates in the mosquito stages would make it harder to interfere with folate metabolism to block malaria transmission. Similarly, the ADCS KO parasites were able to complete liver-stage development normally and cause blood-stage infections in mice without extra *p*ABA supplementation. This is likely due to a rich supply of folates concentrated in animal livers, which renders active *p*ABA *de novo* synthesis nonessential in the parasites.

The major conclusion from this study [1] is that *Plasmodium* parasites use a combination of salvage and synthesis to ensure an adequate supply of *p*ABA (and folates) to support their fast growth in the asexual blood stages. Yet, a few key questions remain to be answered. Does *p*ABA synthesis fluctuate according to the quantity of *p*ABA or related precursors available for salvage? In animal models, can we develop methods to completely restrict *p*ABA salvage sources from diets and the host's microbiota? If *p*ABA salvage is indeed the main supply route over the entire life cycle, why have the enzymes of the *p*ABA *de novo* synthesis pathway not been abandoned during evolution as an unnecessary waste of resources, as they have been in mammals?

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https://doi.org/10.1016/j.pt.2019.01.010

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Forum

Fighting Cancer Using an Oncofetal Glycosaminoglycan-Binding Protein from Malaria Parasites

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Malaria research has led to the discovery of oncofetal chondroitin sulfate, which appears to be shared between placental trophoblasts and cancer cells and can be detected by the evolutionary refined malaria protein VAR2CSA. Interestingly, using recombinant VAR2CSA to target oncofetal chondroitin sulfate shows promise for novel cancer diagnostics and therapeutics.

The Oncofetal Hypothesis: Trophoblasts and Cancer Cells

Cancer is a leading cause of morbidity and mortality. The disease covers a group of more than a hundred different cancer types all sharing the feature of abnormal and uncontrolled cell growth [1]. Strategies for cancer diagnostics and therapeutics are often based on targeting nonexclusive but overexpressed markers in tumor tissue or body fluids, which limits the sensitivity and specificity for diagnostics and leads to various severe side effects during cancer treatment. In the quest of improving cancer outcomes, decades of research have focused on the identification of a universal but specific magic bullet to target all cancers.

The search for an omnipresent cancer target has included oncofetal markers. Such markers will be present during fetal or placental development but reduced to nondetectable levels in matured tissue. The expression of an ideal oncofetal marker reappears in a broad repertoire of tumor tissue, whereas it remains dormant in nonmalignant tissue. The search for oncofetal targets dates back to 1902, when the embryologist John Beard first proposed the trophoblastic theory of cancer (Box 1) [2]. Even though Beard considered cancer to arise from the germ cells, he described the cancer cells as similar to trophoblasts. In this regard, special attention has been drawn to the placenta. The majority of the cells forming the placenta are trophoblasts. During early embryonic development, these cells rapidly multiply and create their own blood supply. Furthermore, they invade the surrounding maternal tissue and resist immune surveillance. Interestingly, all of these trophoblastic characteristics are also essential traits of cancer cells. Regardless of whether Beard's hypothesis conforms to reality, the phenotypic comparison of cancer cells with trophoblasts remains intriguing.