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Indispensible genes in malaria A critical assessment of new opportunities for drug discovery to treat malaria

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Malaria parasites, carried by mosquitos and transmitted to humans, infect ~200 million individuals and cause ~500,000 deaths each year (1). 15 years after identifying the genome sequence of a malaria-causing parasite, *Plasmodium falciparum*, malaria treatments still rely heavily on chemicals derived from natural products that were used centuries ago (2). With cell-based functional assays, the gap between genome-sequences of *Plasmodium* sp. and the identification of valuable new therapeutics may be reduced by determining which genes are essential for parasite propagation in the disease-causing blood-stage forms of human malaria. The most potent, clinically useful antimalarial drugs rapidly eliminate parasites growing in red blood cells (RBCs). On page XXX of this issue, Zhang et al. (3) report a mutagenesis screen on *P. falciparum* cultured in human RBCs, identifying 2,680 indispensable parasite genes. This is potentially important for drug discovery because many antimalarial drugs are known to inhibit essential gene products of parasites (2). However, it is important to critically assess what fraction of these essential parasite genes will be good drug targets and how should one prioritize such targets for drug discovery.

The technical effort, patience, and care required to identify essential genes for the blood-stage forms of human malaria parasites cannot be overstated. Even the most experimentally tractable species of human malaria parasites, *P. falciparum*, grows ~1,000 times slower than other micro-organisms such as *E. coli*. Previous efforts to identify essential genes, through random insertions of disabling pieces of DNA into malaria parasite genes were inefficient, with success rates near one per million parasites in culture. The piggy-back transposition mutagenesis system used by Zhang et al. allows for at least one insertion (mutation) in a random location per parasite genome (3). Combining this controlled mass mutagenesis with parasite pooling strategies, deep DNA sequencing, and bioinformatics, Zhang et al. now provide a reliable list of non-essential genes. When insertions occur in non-essential genes parasites grow successfully. Essential genes are inferred from genes lacking any mutations in growing parasites. It is assumed that parasites with mutations in essential genes would not grow and not survive the screening process.

Zhang et. al. find that of 5,380 malaria genes, nearly 50% are essential for growth in the blood-stage of the malaria parasite life-cycle (see the figure). This estimate of essential genes may also apply to other species of human malaria. Interestingly, a distant mouse malaria parasite (*P. bergeri*), which does not infect humans, has a high fraction of essential

genes for growth in RBCs (4). Within the list of essential *P. falciparum* genes may lie our best hopes for identifying good targets for the most clinically relevant part of the parasite life cycle. Even if the malaria research community, within a decade or two, finds that only 10% of the 2,680 identified essential malaria genes are high-value targets for drug-development, this screening approach will be considered successful.

There are several reasons for setting modest expectations. The essentiality of a gene is not likely to be sufficient for the gene product to be a high-value target for cellular pharmacology. High-value drug targets are truly rare. Global small-molecule screens involving more than 2 million different drug-like organic compounds directed at blood-stage malaria parasites have identified very few new druggable targets (2, 5, 6), compared to the number of essential genes we now know of (3) and compared to genes known to be actively expressed in human malaria parasites (7, 8). Furthermore, away from cell-based studies, even small chemical libraries directed at single purified protein targets routinely generate dozens of potent inhibitors (2, 6). In parasite cell assays, not only are good inhibitors rare but many structurally distinct potent inhibitors of parasite cell proliferation converge on the same ~12 targets (6, 9), most of which have already been identified.

Furthermore, not all enzymes even in essential metabolic pathways are equally druggable. In the essential, linear, pyrimidine biosynthesis pathway in malaria parasites, only a few enzymes are good targets in an intact cell (10, 11).

Finally, to be prioritized for drug development, a small-molecule inhibitor must rapidly kill parasites probably without achieving total inhibition of target activity. Inside a cell, even a potent enzyme inhibitor faces competition from accumulating substrates, and from synthesis of replacement target proteins. Select enzyme targets do trigger cell-death even after partial inhibition. Such targets repeatedly appear as high-value druggable targets, regardless of whether one is interrogating parasites, bacteria, or cancer cells. For example, the nucleotide synthesis-supporting enzyme dihydrofolate reductase (DHFR) is a proven target in the treatment of malaria (by the drugs pyrimethamine and proguanil), bacterial infection (with the antibiotic trimethoprim), and cancer (with the chemotherapeutic methotrexate, which is also an immunosuppressant used to treat autoimmune diseases) (12). These cells are also highly vulnerable to inhibitors of metabolically related thymidylate synthase (TS). It is now understood that even partial inhibition of DHFR or TS leads to a buildup of the nucleotides deoxyuridine monophosphate (dUMP) and deoxyuridine triphosphate (dUTP), and incorporation of unwanted uridine residues into DNA, DNA strand-fragmentation, and cell death (13). In malaria parasites, inhibitors of DHFR or TS act selectively, partly due to host-parasite variations in active sites of the target enzyme but also partly due to parasite-specific variations in regulatory responses to such inhibitors (14). As parasites become highly resistant to existing drugs, such as pyrimethamine, the hunt for new high-value targets becomes more important.

Overall, the study of Zhang et al. offers a powerful start for identifying rare, high-value potentially druggable processes in human malaria parasite. It will inspire other complementary analysis of the data and also new functional screens. For instance, detailed bioinformatics will reveal which essential genes are unique to parasite biology and, later if

found druggable, they will offer clearer paths to selective and safe pharmacology. The extension of insertional mutagenesis screens to other stages of the parasite life-cycle, beyond the blood stage, should help generate inhibitors suited for broader community-wide preventative malaria campaigns that control the disease before there are clinical symptoms. Improvements in conditional CRISPR-dCAS, and related genomic technologies which allow down-regulation of specific genes, without cutting DNA, should help identify genes that trigger parasite death after even partial loss of target activity (15). For these reasons, continued evolution of malaria genomic tools is expected, which will accelerate discovery of high-value drug targets in the parasite genome.

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TOTAL GENES ⁽³⁾	STAGE	CLINICAL SYMPTOMS	TRANSCRIPTS IDENTIFIED ^(7,8)	PROTEINS EXPECTED ^(7,8)	ESSENTIAL GENES ⁽³⁾	POTENT SELECTIVE INHIBITORS ^(2,5)	TARGET-INHIBITOR PAIRS ^(6,9)
5,438	A. RBC	Yes	4,871	2,415	2,680	250	25
	B. Gametocyte	-	4,478	2,270	?	5	3
	C. Liver	-	1,985	712	?	10	5

A highly active malaria genome reveals many essential genes but few good drug targets. Malaria parasites activate a large part of their genome in every life cycle stage, but high-throughput screens with millions of small molecules reveal very few “druggable” targets. Numbers in this table are approximation from references cited in the top row. Adobe stock photo (mosquito) was used in the generation of the illustration. The following restrictions apply:

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