lated aptamer is a logical progression from parallel innovations in the selection and, occasionally, rational design of ligand-regulated ribozymes and DNAzymes, a number of which have been described in the literature over the past few years. The first convincingly allosteric ribozyme (a hammerhead ribozyme whose activity was modulated by the binding of the small molecule adenosine) was generated by rational design by Tang and Breaker [7]. Subsequently, however, many different small molecule ligand-regulated variants of both naturally occurring and in vitro selected ribozymes and DNAzymes have been described and are discussed in the recent review by Breaker [8]. The challenge for researchers producing both ligand-activated ribozymes and ligand-activated aptamers is now to demonstrate that the level of control that can be exercised with these promising reagents in vitro will also be reflected in intracellular environments.

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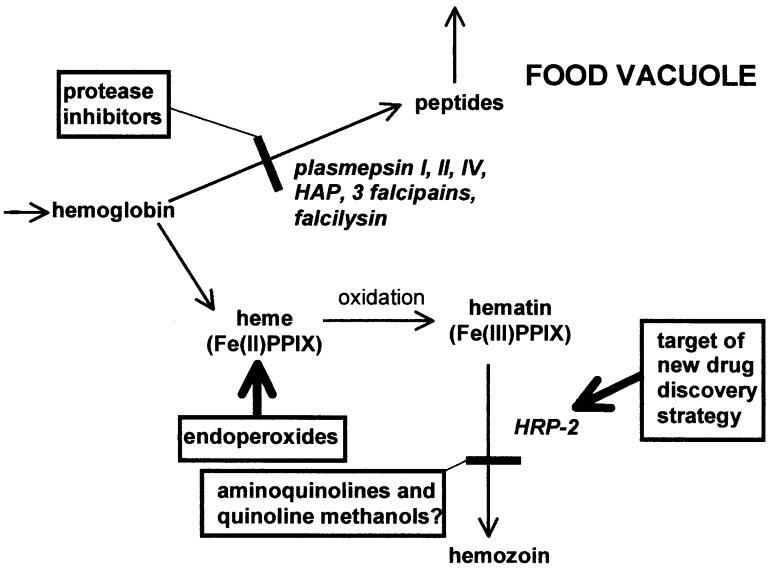
Discovering Antimalarials: a New Strategy

Recent discoveries have uncovered some key processes that occur in the food vacuole of the malarial parasite. Consequently, new families of potential antimalarials that inhibit HRP-2, a hitherto unexplored drug target, were identified using a novel screening method.

Malaria is one of the world's leading killer infectious diseases. Although almost a third of the Earth's population is considered to be at risk from this disease, about 90% of infections and deaths occur in Africa [1], contributing significantly to underdevelopment and poverty on this continent [2]. The reasons for the current severity of the malaria problem are multifaceted, but among them is the occurrence of drug-resistant strains of parasite. Most notable in this regard is parasite resistance to chloroquine, which is now almost universal. In the past, this drug, having several excellent properties, became the mainstay of treatment and was a key component of malaria control strategies. In particular, chloroquine was highly effective against the parasite, had very few adverse side effects, was safe for use in pregnancy and young children, and was very cheap [3]. Its loss has been a major setback [4]. The challenge now is to find new compounds with antimalarial activity that can be developed into drugs that are cheap enough for use in poor third world countries.

A positive development over the last decade has been the considerable increase in our understanding of processes occurring within the parasite that are relevant to the mode of action of current antimalarials and which provide targets or potential targets for new antimalarial compounds. The causative agents of malaria are protozoal parasites of the genus *Plasmodium* (with *P. falciparum* the cause of fatal cases) [5]. They have a complex life cycle involving liver and blood stages in the human host, where asexual reproduction occurs, and a stage in the vector mosquito (*Anopheles* genus), where sexual reproduction occurs [5]. Symptoms and pathology are associated entirely with the blood stage, during which the parasite is located within the red blood cell of the host [5]. During this part of the life cycle, the parasite ingests hemoglobin into a specialized acidic compartment called a food vacuole. The hemoglobin is proteolytically digested into small peptides that ultimately supply the parasite with amino acids [6] (although intriguingly, these nutrients appear to be oversupplied [7]). Proteolysis is carried out by four aspartic proteases, namely plasmepsins I, II, and IV, and histo-aspartic protease (HAP) [8], three cysteine proteases (falcipains) [9], and a zinc protease (falcilysin) [10]. All of these represent potential targets for antimalarials (see Figure) and are currently the subject of intense investigation. Digestion of hemoglobin releases heme [iron(II)protoporphyrin IX, Fe(II)PPIX] into the food vacuole, where it is oxidized to hematin [H_2O-Fe(III)PPIX] [6]. Heme is another possible drug target and has been implicated in the mode of action of endoperoxide antimalarials, such as artemesinin, which have been proposed to form radical adducts with heme that act against the parasite [11]. Hematin is believed to be the target of chloroquine and other quinoline antimalarials, and there is evidence suggesting that these drugs act by preventing the detoxification of hematin (see Figure) [12], essentially all of which is normally converted to a very insoluble microcrystalline dimer of Fe(III)PPIX called hemozoin (or malaria pigment) [13]. Chemically, hemozoin is identical in composition [14] and structure [15] to β-hematin, a synthetic product that can easily be prepared from a solution of hematin.

The mechanism of hemozoin formation in the parasite



Drug Targets in the Food Vacuole of the Malaria Parasite

Key pathways in the degradation of hemoglobin and the detoxification of heme are shown. Bold arrows and bars indicate drug targets, with drug classes shown in boxes. Protein targets are given in italics. HRP-2 (histidine-rich protein 2) is believed to initiate or catalyze hemozoin formation and is the target of a new strategy for antimalarial drug discovery reported in this issue of *Chemistry & Biology* [20].

is not well understood, but histidine-rich protein 2 (HRP-2) has been implicated as an enzyme or, more likely, an initiator in this process [16]. HRP-2 specifically binds about 50 Fe(III)PPIX molecules per protein as bis-histidyl complexes, with β-hematin formation commencing only after the hematin binding sites on the protein are saturated [17]. Drugs such as chloroquine have variously been proposed to inhibit hemozoin formation via direct interaction with hematin [18], by displacing hematin from HRP-2 [19], or by preventing its binding to this protein. Irrespective of which of these hypotheses are correct, HRP-2 represents a hitherto unexplored target for new antimalarials (see Figure). In this issue of *Chemistry & Biology*, the development of a new rapid-throughput screening method for investigating the ability of compounds to prevent hematin binding to HRP-2 is reported [20]. This method has the advantages of speed and ease of application for searching for compounds that have potential antimalarial activity. Using this novel technique, Marletta and coworkers [20] investigate a combinatorial library of 35 compounds. The authors find that activities of these compounds are strongly correlated with their ability to inhibit hematin binding to HRP-2. In addition, several compounds show excellent activity against cultured parasites, including a chloroquine-resistant strain.

The significance of the work lies in the development of a new, simple, and cheap screening method, the explicit selection of compounds targeted for the first time against hematin binding to HRP-2, and the identification of new families of compounds that show strong activity against cultured malaria parasites, including chloroquine-resistant parasites. These discoveries may be elaborated on in the future to yield new drugs for treatment of this devastating disease.

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