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Review

(Special Topic)

Enlistment of omics technologies in the fight against malaria: Panacea or Pandora's Box?

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Plasmodium spp, the causative agent of malaria, imposes an enormous cost on the developing world. Current methods are inadequate for long-term management and eradication, and new treatments are desperately needed. The modern arsenal of "omics" technologies appears to offer a promising approach to engineering a long-term solution to malaria. However, because funding for malaria research is chronically limited, the potential results of omics methodologies must be examined to address whether the investment is justified. This review provides an overview of a suite of omics-related technologies in terms of their potential contribution to the field of malaria research. © Pesticide Science Society of Japan

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Introduction

Malaria is an ancient human disease caused by an apicomplexan parasite that has coevolved over the course of millennia to require both a vertebrate and an invertebrate host in an intricate three-way interaction.[1,2)] Of the four *Plasmodium* species that affect humans, *Plasmodium falciparum* is responsible for the most virulent form of malaria and is considered one of the world's most pathogenic microbes.[3)] Throughout its life cycle the parasite progresses through a series of distinct sexual, invasive, and replicative stages (Fig. 1). When biting an infected human host, the mosquito takes in gametocytes as part of the blood meal.[4)] Within the midgut, male and female gametocytes fuse, forming oocysts which then bud to become sporozoites.[4)] The highly mobile sporozoites migrate to the salivary gland where they are poised to invade a new human host during a subsequent bloodmeal.[3)] Once in a human host, sporozoites migrate to the liver and invade hepatocytes, where they replicate and release thousands of merozoites into the blood.[3)] Merozoites invade erythrocytes where they differentiate into trophozoites and begin a cycle of replication, explosive release,[5)] and reinvasion, leading to the periodic nature of malaria symptoms.[3)] Periodically, some merozoites differentiate into male and female gametocytes for transmission through the mosquito host, thereby completing the complicated three-way life cycle shown in Fig. 1.[3)]

A range of complementary intervention strategies to control the parasite is currently in use, including mosquito habitat disruption, domestic insecticide application, insecticide-treated bed nets, anti-malarial drugs, *etc.*[2)] Generally these approaches are reasonably successful and cost-effective[6,7)]; for example, long-lasting insecticide-treated bed nets can remain effective without re-treatment following six years of use.[8)] However, a resurgence in the disease is being seen in many parts of the world, due in part to increasing resistance to anti-malarials and insecticides.[9)] The number of global incidents has reached ~515 million per year, a 92% increase compared to the WHO's estimate of 278 million in 1998.[9)] Particularly in light of these developing problems, funding continues to be a limiting factor in malaria management, and budgets for malaria control consistently fall short of what is required, perhaps by an order of magnitude.[6,10)] A more thorough understanding of the parasite's biology may lead to a more focused and effective use of malaria research funding.[11,12)] Though the

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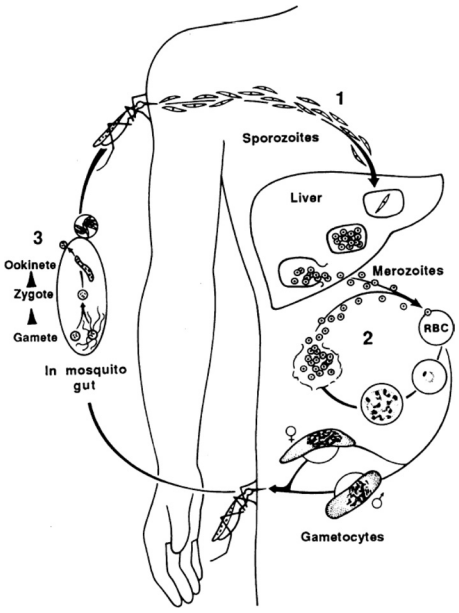

Fig. 1. Life cycle of the malaria parasite. 1) Sporozoites: female anopholine mosquitoes, while they ingest a blood meal, inoculate sporozoites into the bloodstream that rapidly invade hepatic cells. Hepatic schizonts: One sporozoite develops into 20,000 merozoites within a hepatic cell. Merozoites rupture from hepatic cells and pour into the bloodstream to invade erythrocytes (RBC). 2) Asexual erythrocytic cycle: Asexual parasites mature within erythrocytes from rings to schizonts in 48 to 72 hr, the time varying with the malaria species. Disease and death in malaria is caused by this stage of the life cycle. 3) Cycle in mosquito: Some red cell parasites differentiate to gametocytes, which infect mosquitoes. Fertilization in the mosquito midgut occurs rapidly. Within 24 hr zygotes transform into ookinetes which penetrate the midgut to form oocysts and, later, sporozoites. Reprinted with permission from Miller *et al.*: *Science* **234**, 1349–1356 (1986). Copyright 1986 AAAS.

complexity of the system poses daunting technical challenges, it may well be the intricacy of this three-way interaction itself that is the parasite's Achilles heel.[13)]

Conventional technologies are poorly suited to identifying drug targets through comprehensive analyses of an organism's biology and drug mechanisms.[11,14)] A promising alternative involves applying the suite of "omics" technologies, *e.g.*, genomics, transcriptomics, proteomics, metabolomics, and glycomics, to develop a multi-faceted profile of the parasite's inner workings in an effort to locate its most vulnerable points. The "omics revolution" has fostered an ambitious, data-intensive approach to biology that emphasizes large-scale, aggregate studies using automation, high-throughput techniques, and sophisticated data mining methodologies. Initially these technologies were characterized as being prohibitively expensive and time-consuming with long delays in the return on investment,[15)] but technological advances and commoditization have made these technologies more widely accessible and affordable.[16)] However, it can be argued that available malaria funding could instead be used to augment traditional interventions to achieve more immediate results.[17)] Given this tradeoff, it is important to evaluate whether this type of research is an appropriate investment for limited resources to control the malaria parasite.

Genomics

Genomics, the original and quintessential omics application, employs DNA sequencing, assembly, and annotation to document the genome of an organism in its entirety.[18)] In an ambitious project spanning seven years and at a cost of more than US$20 million, three international research centers participated in a joint effort to sequence the complete *P. falciparum* genome.[19,20)] While some work remains,[21)] essentially complete genomes of all three organisms involved in the parasite's complex lifecycle are now available.[22)] Many other sequencing projects are currently in progress, including a number of apicomplexan species,[23,24)] other *Plasmodium* species[25–27)] and additional strains of *P. falciparum.*[26)] These additional sequence projects should contribute substantially to comparative genomics efforts and may help to indicate potential new drug targets as well as provide information on fundamental biological processes in apicomplexan parasites.

An important point in evaluating the investment in sequencing projects is that each project may in turn reduce the cost and duration of successive projects. Skills and knowledge invested during the initial *Plasmodium* sequencing project transfer directly to additional sequencing projects.[28)] *Plasmodium* has an extreme 80% AT bias in nucleotide composition[20)] and is consequently difficult to sequence and assemble.[29)] Because innovative approaches had to be developed to overcome these obstacles, follow-up sequencing efforts should be completed quicker and with reduced cost relative to the initial sequencing project.[28,30)] A haplotype map covering multiple strains is being developed and will be a significant aid in understanding the factors contributing to parasite fitness and survival.[31)] The acquisition of a whole genome also enables genome-wide genotyping of unsequenced isolates through microarray assays.[32)] In this way, duplication of genes and gene clusters likely to play a role in parasite invasion and growth can be identified.

Comparative genomics with other *Plasmodium* species suggests that genes in the chromosomal cores are relatively conserved, whereas genes in the subtelomeric regions are highly species-specific.[33,34)] This may be because subtelomeric regions are generally unstable and are associated with a high rate of recombination as well as nucleotide loss and disruption.[35)] Genes in this region are often involved in host–parasite interactions (*e.g.* invasion[36)]) and may therefore trigger host immune responses, perhaps leading to diversifying selection as a result of immune pressure.[33,37,38)] In fact, several aspects of the parasite's biology appear to promote genetic diversity, including secondary loss of DNA repair proteins, suggesting that proofreading errors may be more common in

P. falciparum than in other eukaryotes, allowing rapid adaptation.[39]

Due to its association with parasite virulence and immune evasion, the *var* gene family encoding the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) surface protein is one of the most well-studied subtelomeric gene families.[40] Located mainly in the variable region on the chromosomal peripheries, *var* genes undergo recombination at a rate eight times higher than average.[41,42] Furthermore, genes of this family appear to undergo inter-chromosomal recombination as well, leading to a diverse repertoire of antigenic genes.[41,43] Genomics can help reveal the extent of antigenic variation in this family, thereby aiding the goal of discovering broadly representative patterns that can be targeted in new interventions.

Sequencing of the *P. falciparum* genome revealed that the *var* genes could be organized into five subgroups depending on the upstream flanking sequence (UpsA to UpsE). This information will be pivotal in understanding how *var* gene transcription is regulated.[20,44] There is evidence that the members of a group are co-regulated,[45,46] which implies that they differ in function. Since the upstream groups also correspond to downstream sequence properties, investigators will be able to track parasite attributes associated with these groups. For instance, UpsA *var* genes in the 3D7 genome encode PfEMP1 proteins with fewer cysteine residues in the first DBL domain.[47] Studies from other isolates corroborate this observation,[48] and this *var* group is implicated in severe malaria.[49–51] Classification of the *var* genes has also enabled researchers to single out unique *var* genes that fall outside the main groups. For example, during pregnancy-associated malaria var2CSA is believed to be the principal ligand that binds to chondroitin sulfate A in the placental endothelial lining.[52,53] This type of knowledge could prove to be useful in identifying stage-specific malaria vaccine targets.

Transcriptomics

Whereas genomics reveals the organization and composition of an organism's genome, but not the conditions under which individual genes are expressed, transcriptomics is used to identify patterns of gene expression by detecting differences in mRNA levels between varying experimental conditions. Just as shotgun sequencing methods have made large-scale sequencing projects possible, high-throughput technologies such as microarrays make it feasible to collect huge gene expression datasets quickly, accurately, and reproducibly.[16]

Microarray expression data have provided useful insights into the parasite life cycle, indicating possible functions for hypothetical proteins throughout the life cycle[54,55] and revealing an unusual cascade of transcriptional regulation.[56] In fact, there are many unassigned genes in *P. falciparum*, and many aspects of gene regulation in *P. falciparum* are unusual and poorly understood,[38,39,54,57] *e.g.* extensive histone modification[58] and combinatorial gene regulation.[59] Even though 88% of all predicted genes are expressed at least once in the life cycle, and more than half appear to be constitutively expressed,[60] the function of 60% of the predicted open reading frames is unknown, suggesting an important ongoing role for transcriptomics in malaria research.[60]

Because of the large number of unassigned genes, transcriptomics techniques can be useful in identifying stage and host-specific genes and signaling pathways.[12,61] The asexual blood stages are fully responsible for the clinical symptoms of malaria, and transcriptomic techniques have revealed a coordinated program of gene expression during intraerythrocytic development that should help pinpoint genes critical to this pathway.[62] The sexual stage, however, is required for transmission to the mosquito host,[63] and the parasite undergoes sexual differentiation and expresses gametocyte and sex-specific genes.[56,64] Therefore, it might be possible to block transmission of the disease by disrupting sexual stage-specific gene regulation.[55,61,63]

Microarray data have also demonstrated up-regulation of genes involved in immune evasion, shedding light on potential targets associated with host–pathogen interactions.[65,66] Disease severity is determined in part by the parasite's ability to evade immune recognition and splenic clearance, which is heavily influenced by which *var* gene is expressed.[67,68] Therefore, it is crucial to understand the mechanism by which the parasite determines expression patterns of individual *var* genes.[67] Only one *var* gene is expressed at any given time in the mature trophozoite, while the rest are suppressed through an epigenetic gene silencing mechanism; however, from one cycle to the next, the parasite may switch which *var* gene is expressed.[45,67,69,70]

In immune-naïve patients, some highly virulent PfEMP1 variants appear to confer greater transmission success, but continuous exposure to the immune system appears to constrain expression of these genes.[71] Because this phenomenon suggests a mechanism for acquired natural immunity, better understanding may provide important insights for vaccine development. For example, as mentioned above, a subset of *var* genes coding for high binding affinity to chondroitin sulfate A, a glycan found most abundantly in the placenta, appears to be preferentially expressed in women in *prima gravidae.*[72] Perhaps due to the restricted antigenic variation within this subgroup, natural immunity appears to develop more rapidly in this form of malaria, suggesting that this class of PfEMP1 may yield a promising vaccine target for pregnancy-associated malaria.[73,74]

Proteomics

Numerous studies have shown the lack of a correlation between the expression patterns of mRNA and the downstream proteome both in vertebrates and invertebrates.[75,76] These discrepancies are partly due to a variable time lag between mRNA transcription and the subsequent translation, a time lag potentially enhanced by post-transcriptional regulation of

protein expression.[27,77] Accordingly, quantitative alterations in the transcriptome are merely suggestive of what may happen in a cell or a tissue at a certain time point in response to a given stimuli. To this end, the importance of studying the downstream protein expression in concert with genomics approaches quickly became evident in the post-genomic era. Consequently proteomics, defined as the quantitative or qualitative determination of alterations in abundances or modifications of specific proteins in response to a stimulus, emerged along with a range of proteomics methods. Over the past decade, these have matured into primarily two lines of high throughput proteomics methods, based on the separation method utilized: In gel-based proteomics, the mixture of intact proteins are separated in a polyacrylamide gel according to charge in one dimension, and according to molecular weight in a second dimension, referred to as 2-dimensional gel electrophoresis (2DE). As a result, each protein species is focused as a spot in the 2DE gel. Following quantification, the protein spot is excised, digested into peptide fragments, and analyzed by mass spectrometry (MS). In shotgun proteomics, the proteins are digested into peptide fragments prior to separation, and the complex mixture of peptides is then separated through liquid chromatography (LC) prior to identification and quantification by MS analysis. Recently, proteomics has established itself as a vital part of both development and validation of novel treatments and vaccines against malaria. A convincing example demonstrating the strengths of proteomics analyses is illustrated by expression analyses performed on the PfEMP1 *var* gene.[78] While RT-PCR analysis of cases of placental malaria clearly demonstrated that the COMMON *var* gene was expressed, subsequent western-blot analysis showed that in reality, a different *var*-gene product with a much lower molecular weight was expressed.[79] Given the importance of selective *var*-gene expression in the parasite's ability to evade the host immune defense, analysis of the transcriptome alone can be misleading, and could lead to incorrect assumptions about disease severity if utilized in a diagnostic purpose.

The completed sequencing of the malaria parasite has provided the framework necessary to facilitate global analysis of the expression patterns of the parasite proteome. In one of the pioneering studies by Florens *et al.*, 2415 proteins representing 46% of the *Plasmodium* proteome were identified.[80] The study revealed a substantial proteome specificity between the four life stages analyzed (sporozoite, trophozoite, merozoite and gametocyte), and only 6% of the identified proteins were expressed in all four stages. More importantly, over 50% of the detected proteins were identified as hypothetical proteins, which lack homology with any known proteins in other species. The large number of potentially *Plasmodium*-specific proteins affords new hope in the hunt for a safe and efficacious vaccine against malaria. In a concurrent study, Lasonder *et al.* identified 1289 proteins from a different strain of *P. falciparum*, of which 575 were expressed exclusively in the sexual stages specific to the life stages in the mosquito host.[81] The findings from these two studies gave credibility and momentum to the use of proteomics applications in malaria research. A large number of studies probing the global alterations in the *Plasmodium* proteome between the different life stages of the parasite has followed over the last few years (for detailed review, see ref. 82–84).

One of the main limitations in both lines of high throughput proteomics has traditionally been a poor success rate in the analysis of membrane associated proteins. Due to their high hydrophobicity, these proteins have proven difficult to separate both by 2DE and LC. Yet, membrane proteins expressed by the various blood stages of the parasite, both on the invasive sporozoite and merozoite life-stages of the parasite itself, and those expressed on the surface of invaded host erythrocytes during the trophozoite life stage, have received considerable attention as putative pharmaceutical targets both due to their importance in host cell invasion, and due to their accessibility in terms of drug delivery. In spite of the limitation in current proteomics methods, successful investigation of several membrane proteins of pharmacological interest, including PfEMP1, has been performed through proteomics applications. Novel methods for investigation of the detergent-resistant membrane fraction of the mature blood-stage parasite resulted in identification of proteins associated with the rhoptry organelle, multiple-membrane spanning proteins and proteins that are exported to the erythrocyte cytosol.[85] The rhoptry organelle plays an essential part in invasion of host cells, and rhoptry-specific proteins thus represent putative targets in the development of both pharmaceutical therapies and vaccines against malaria. Proteomics studies of a related parasite from the same phylum as *Plasmodium* identified 38 novel proteins located to the rhoptry organelle.[86] Furthermore, the protease Falcipain 1 has been shown through chemical proteome screening to be directly involved in parasite invasion of host erythrocytes.[87]

In addition to rhoptry-organelle associated proteins, glycosylphosphatidylinositol (GPI)-anchored proteins have been found to be enriched in the membrane fraction.[85] GPI-anchored proteins, which coat the surface of *P. falciparum* merozoites, are putative targets for a blood-stage malaria vaccine. Three surface proteins containing a 6 cysteine repeat were of particular interest, as this repeat has also been associated with surface adhesion in other life stages of the parasite. All three proteins were recognized by antibodies present in infected individuals.[85] Utilization of tritiated glucosamine lead to identification of an additional GPI-anchored proteins making up over 90% of the GPI-anchored schizont/merozoite proteome.[88] Two of these protein species, merozoite surface protein (MSP)-1 and -2, were estimated to make up two thirds of the membrane coat of the parasite in this life stage. In an alternative method for identification of membrane-spanning proteins, trypsination of the erythrocyte surface followed by mass spectrometric analysis revealed a novel group of pro-

teins, termed surfins, expressed both on the infected erythrocyte and on the merozoite.[79)]

During the trophozoite life stage, the parasite constructs an elaborate protein transport system to export proteins to the erythrocyte surface.[89)] Since the erythrocyte is the only non-antigen-presenting cell type in the blood stream, the parasite utilizes a unique method to evade the host's immune system by exporting its own proteins to the erythrocyte surface. Through immunoprecipitation followed by shotgun proteomics, Sam-Yellowe and colleagues identified a novel gene family that is expressed simultaneously as the *rif* gene products during the trophozoite life stage.[90)] These membrane-spanning proteins are localized to the Maurer's cleft,[90)] which is involved in protein transport from the invading parasite to the cytosol of the host erythrocyte.

The majority of the proteomics studies published to date have been performed on blood stage parasites, primarily on *in vitro* systems. The liver stages, which are of equal interest from a pharmaceutical standpoint, have received less attention. The shortage of proteomics investigations of the liver stages are likely due to the lack of available *in vitro* models,[82)] and *in vivo* animal models might be required. Korir *et al.* recently utilized proteomics approaches to verify extensive sequence homology in the externally exposed regions of *Plasmodium knowlesi* schizont-infected cell agglutination (SICA) antigens with the corresponding *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) antigens.[91)] The high homology of this important extracellular region validates the use of the Rhesus animal model for the study of this family of surface proteins important in cell adhesion. Furthermore, *var* genes have also been shown to be expressed in the sporozoite stage.[80)] The sporozoite life stage is of potential interest in pharmaceutical applications, particularly if targeted while still in the blood stream, prior to its invasion of host hepatocytes. However, little is known about this proteome, and further investigation is required.

To summarize, a number of proteins uniquely expressed in a specific life stage have been identified through the use of proteomics. Proteomics analysis has revealed that a vast number of these gene products are not expressed in the host organism, which makes them promising as potential parasite-specific drug targets. Proteomics analyses are also instrumental in validating predictions regarding the *Plasmodium* proteome generated using bioinformatics tools,[82)] and to further elucidate the mechanisms of action of currently used antimalarial agents, such as quinolines[92)] and CoArtem.[93)] Given that proteomics still is a relatively new methodology with great potential for improvement, particularly in regards to membrane spanning proteins, the prospects of providing new insight into the pathology and pharmacology of malaria through proteome analysis are indeed promising.

Metabolomics

Metabolism is a key aspect of phenotype and consequently the next logical step in functional genomics is to describe the distribution of metabolites in an organism following treatment or perturbation.[94)] Metabolomics is a research approach that aims to identify and quantify the metabolome, which can be defined as the dynamic set of all small molecules present in a biological sample or organism under a given set of physiological or environmental conditions.[95)] While analytically challenging due to the sheer volume and chemical diversity of the compounds in the metabolome, existing analytical platforms, including mass spectrometry, NMR and chromatographic separation systems are capable of producing quantitative data on numerous metabolites simultaneously. Given the complexities in studying the biology of the malaria parasite, metabolomic investigations could prove to be very elucidating. To date, no in-depth metabolomics studies have been performed on the malaria parasite; however, a number of different groups have discussed the utility of a systems biology approach to discover new networks and interactions in the parasite.[32,83,96,97)] Metabolomics data are an important component of a systems biology analysis, especially when examining host–pathogen interactions.[97)]

There are a number of topics that lend themselves particularly well to metabolomics-based investigations. One of the key thrusts of current malaria research is to identify new therapies, including drugs and vaccines. However, as discussed above, the complex life cycle of the malaria parasite is challenging to study. Genomic and transcriptomic studies have been very useful in investigating the expression profile in the genome and to predict the function of uncharacterized genes.[20,64)] However, questions still remain in terms of conclusively identifying gene function, and current methods are dependent upon examining correlations in gene-expression profiles or co-regulation in transcription.[32)] Similar limitations have been observed in proteomic studies, as a significant number of the identified proteins have unclear function.[80,81)] Therefore the acquisition of metabolomics data on parasite biochemical processes could provide an extremely useful complement to existing data sets and increase our ability to identify both gene and protein function.

Classical drug development paradigms suggest that one should look for targets for which no homologous target exists in the host organism. However, it is more important to locate a target that is essential for parasite viability. Metabolomics would be particularly effective at this type of research approach. A prime target for a focused metabolomics study in malaria is lipid metabolism, which has been examined in a number of different reviews.[98–101)] Ongoing drug development programs are exploring targets in lipid metabolism for development of antimalarials including *de novo* fatty acid synthesis,[101)] sphingolipid metabolism[102)] and lipid posttranslational modifications.[103)] It has been reported that parasites induce significant alterations in lipid parameters and that changes in lipid profiles occur in patients infected by protozoan parasites.[104)] In addition, lipid metabolism has been shown to

be altered in the host by malaria infection and may reflect metabolic complications associated with severe malaria.[105)]

Lipid metabolism in the malaria parasite has not been extensively examined in the literature, with most studies focusing on effects upon the host (*i.e.*, alterations in erythrocyte or plasma lipid profiles following infection). For example, Maguire and Sherman found that phospholipid composition of infected erythrocytes did not vary greatly, but sphingomyelin content decreased by 47% and the cholesterol/phospholipid ratio showed a 55% reduction.[106)] Holz *et al.* found that octadecenoic fatty acids were elevated over control values in the major phospholipid classes of infected erythrocytes.[107)] Beach *et al.* examined the lipid composition of *Plasmodium lophurae* and found significant differences between the parasite and infected erythrocytes.[108)] The fact that the parasite is able to incorporate lipids from the host suggests that lipid-based metabolomics studies should focus on host–pathogen interactions as suggested by Forst.[97)] Given the importance of lipid metabolism, a lipidomics, or focused-metabolomics approach in lipid profiling, would be useful in further elucidating fluctuations in lipid biochemical pathways and is a logical first-step in metabolomics studies of the malaria parasite.[109)]

Web-based metabolic pathway data on the parasite are already available from a number of sources.[110–112)] These systems are useful for providing information on biochemical pathways and for understanding metabolic flux. However, they lack quantitative information on metabolite concentrations and are therefore of limited ability in studying the effects of perturbations in metabolite levels based upon chemotherapeutic interventions in the parasite lifecycle. One can envision a new layer of data in the PlasmoDB database that provides metabolic information on key metabolic pathways in the parasite in different life stages as well as in the presence of a range of anti-malarial agents. Advances in analytical instrumentation and methods make metabolomics-based studies on the malaria parasite possible. These data would be an excellent complement to existing genomic, transcriptomic and proteomic data on the parasite and could prove to be an important step on the road to true systems biology of the parasite.

Glycomics

Glycomics is an emerging field charged with identifying and characterizing the diverse array of polysaccharide or glycan structures that compose the glycome.[113)] Unlike template-based gene and protein sequences, glycans tend to be nonlinear and diverse and are constructed post-translationally through the activity of a suite of glycosyltransferase enzymes.[113,114)] These differences pose unique challenges for glycomics research. Though still in relatively early stages compared to genomics and proteomics tools, several efforts are underway to develop web databases to integrate available glycan data, including the KEGG Glycan database,[115)] the Consortium for Functional Genomics,[114)] and GLYCOSCIENCES.de.[116)] Scoring matrices have been devised to identify recurring patterns in glycan structure,[117)] and glycosyltransferase expression data can be used to predict the possible repertoire of glycan structures at a specific cell stage,[118)] thereby narrowing the otherwise immense conformational space. In addition, carbohydrate/oligosaccharide microarrays will make it possible to test glycan binding affinity to a number of substrates simultaneously.[113,119,120)] Together with automated oligosaccharide synthesis,[121)] mass fingerprinting tools for oligosaccharide fragment identification,[122)] and glycan alignment software,[123)] these technologies lay the groundwork for a high-throughput glycan analysis approach on a par with current proteomic and transcriptomic approaches.

Glycomics is likely to play an increasingly prominent role in malaria research because glycans and glycan-binding interactions play an important role in parasite virulence. However, standard methods of studying sugar-binding interactions are limited. Like many pathogens,[124)] *P. falciparum* relies heavily on glycans expressed on host cell surfaces for target cell invasion.[125,126)] Glycan binding also contributes to the severity of the disease; infected erythrocytes bind to glycosaminoglycans on vascular endothelial linings to reduce splenic clearance, resulting in impeded circulation and, in severe cases, organ failure, coma, and possibly death.[127)] Similarly, glycan binding is also involved in rosetting, in which PfEMP1 proteins on the erythrocyte surface bind to heparan sulfate and other glycans on uninfected red blood cells to form clusters or "rosettes."[40,128–130)] Rosetting may further impede circulation and is associated with cerebral malaria and other life-threatening forms of the disease.[131,132)] The use of glycomics methods could greatly increase our knowledge of these important interactions and help us understand the mechanisms behind malaria virulence.

Conclusions

Despite the growing investment and interest in malaria research and treatment, there are numerous long-term problems associated with malaria management that remain to be solved. Malaria is fundamentally a disease of poverty, and therefore funding is likely to remain an important limiting factor.[10)] Omics-related research approaches appear to be promising, but funding must be applied effectively to focus on relevant and promising research questions.[17,30)] For example, awareness of sequence data alone is insufficient for developing and evaluating new vaccine and drug targets,[30)] and it is certainly no comfort for those suffering from malaria.[29)] The Wellcome Trust, a major funding source behind malaria research, considers genome research to be the most cost-effective way to support research in infectious diseases.[19)] The genome project may yield 10–20 new drug families, but the bottleneck remains the lack of funding available for testing of existing therapies and interventions.[19)]

A list of key papers in each of the omics areas discussed in this review is given in Table 1. Individually, these different

Table 1. Useful omics-related malaria references

Omics category	Reference	Description
Genomics	Gardner *et al.* 2002[20]	*P. falciparum* genome sequence
	Hall and Carlton 2005[38]	Conclusions drawn from comparative genomics across *Plasmodium* species
	Hall *et al.* 2005[27]	Genomic, transcriptomic, and proteomic analysis of the parasite life cycle
	Kooij *et al.* 2005[34]	Use of a whole-genome synteny map to identify species-specific genes
Transcriptomics	Ben Mamoun *et al.* 2001[62]	Examination of gene expression patterns during the asexual intraerythrocytic stage
	Bozdech *et al.* 2003[56]	Examination of transcriptional regulation during intraerythrocyte development
	Daily *et al.* 2005[66]	*In vivo* gene expression study of surface proteins
	Hayward *et al.* 2000[61]	Comparison of stage-specific gene expression
	Le Roch *et al.* 2003[54]	Identification of gene function by expression profiling
	Llinas *et al.* 2006[64]	Comparative transcriptomics study of three *P. falciparum* strains
	Young *et al.* 2005[55]	Microarray analysis of genes involved in sexual development
Proteomics	Florens *et al.* 2002[80]	Identified 2415 *P. falciparum* proteins from sporozoite, trophozoite, merozoite, and gametocyte life stages
	Lasonder *et al.* 2002[81]	Identified 1289 *P. falciparum* proteins in both human and mosquito hosts
	Belli *et al.* 2005[84]	Analyzed the detergent-resistant membrane fraction of the mature blood-stage parasite
	Gilson *et al.* 2006[88]	Identification of major GPI-anchored proteins in schizont/merozoite proteome
	Winter *et al.* 2005[79]	Discovery of surfin protein family expressed on infected erythrocyte and on the merozoite
	Sam-Yellowe *et al.* 2004[90]	Discovery of novel gene family expressed during trophozoite life stage
	Korir and Galinski 2006[91]	Showed sequence homology between rhesus SICA human PfEMP1 antigen
Metabolomics	Bansal *et al.* 2005[104]	Role of cholesterol in parasitic infections
	Planche *et al.* 2005[105]	Metabolic complications of severe malaria
	Lu *et al.* 2005[101]	Fatty Acid synthesis as a target for antimalarial drug discovery
	Ralph *et al.* 2004[96]	Metabolic maps and functions of the *P. falciparum* apicoplast
	Wenk 2005[109]	Emerging field of lipomics
Glycomics	Hashimoto *et al.* 2005[139]	Glycomics resources in KEGG
	Kawano *et al.* 2005[118]	Glycan structure prediction based on glycosyltransferase expression
	Raman *et al.* 2006[140]	The Consortium for Functional Genomics
	Vogt *et al.* 2003[40]	Identification of heparan sulfate as a mediator of PfEMP1 binding

methods each focus on one aspect of the organism's biology, and may provide useful insights on their own. However, a systems biology approach to combining data collected *via* a range of methods and technologies may provide the ultimate insight into biological processes involved in the malaria infection process and disease progression.[32] Metabolic pathway reconstruction can predict key enzymes and transporters that should be examined as potential new targets[21] as well as reveal species-specific pathways that have an exploitable vulnerability.[133] For example, the parasite appears to lack several proteins that in other eukaryotes are required for metabolism,[134] and it is unable to synthesize several amino acids *de novo.*[39] Similarly, metabolic pathways associated with the apicoplast organelle (a relict plastid (or chloroplast) derived from the endosymbiosis of cyanobacteria that is no longer photosynthetic) may potentially be targeted without interfering with host metabolism.[7,135,136] An omics approach to research questions will increase our ability to exploit these key differences in parasite–host interactions and increase our understanding of the biological processes involved, furthering our progress on the path to a permanent solution.[137] However, it is vital that data from omics-oriented research projects be made freely and quickly available to the international research community.[30] For example, PlasmoDB serves as an interactive clearinghouse to assemble, organize, and cross-reference malaria data from many sources,[138] significantly increasing

the potential impact of omics-related research.

In a paradigm of limited funding resources, directed and focused interventions are required. If the goal is to produce a cure, or at the least to reduce suffering and incidence of disease, then both short and long-term strategies are necessary. Focused applications of limited resources on large-scale data intensive projects are a reasonable investment as long as the data are made freely available. However, significant resources still need to be aliquoted to "front line" interventions so as to ensure a balanced approach in the fight against malaria. The advent of omics technologies has the potential to be a panacea for the devastating effects of the malaria parasite as well as for other infectious diseases. However, the research community needs to avoid the temptations of generating a Pandora's Box of omics research results that consists of a plethora of data, but does not move us closer to a cure.

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