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Leveraging the wheat germ cell-free protein synthesis system to accelerate malaria vaccine development

Bernard N. Kanoi, Hikaru Nagaoka, Masayuki Morita, Takafumi Tsuboi, Eizo Takashima *

Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Ehime 790-8577, Japan

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ABSTRACT

Vaccines against infectious diseases have had great successes in the history of public health. Major breakthroughs have occurred in the development of vaccine-based interventions against viral and bacterial pathogens through the application of classical vaccine design strategies. In contrast the development of a malaria vaccine has been slow. *Plasmodium falciparum* malaria affects millions of people with nearly half of the world population at risk of infection. Decades of dedicated research has taught us that developing an effective vaccine will be time consuming, challenging, and expensive. Nevertheless, recent advancements such as the optimization of robust protein synthesis platforms, high-throughput immunoscreening approaches, reverse vaccinology, structural design of immunogens, lymphocyte repertoire sequencing, and the utilization of artificial intelligence, have renewed the prospects of an accelerated discovery of the key antigens in malaria. A deeper understanding of the major factors underlying the immunological and molecular mechanisms of malaria might provide a comprehensive approach to identifying novel and highly efficacious vaccines. In this review we discuss progress in novel antigen discoveries that leverage on the wheat germ cell-free protein synthesis system (WGCFS) to accelerate malaria vaccine development.

1. Introduction to malaria

Five species of *Plasmodium* cause malaria in humans; namely, *Plasmodium falciparum, P. vivax, P. malariae, P. ovale*, and *P. knowlesi*. After decades of concerted efforts to fight the scourge, malaria remains a disease of global public health importance [1]. *P. falciparum* is the most virulent among the species and puts nearly half of the world population at risk of infection. In 2018, an estimated 93% of the 405,000 malaria associated deaths were in Africa, of which 70% occurred in children under 5 years of age. Despite immense resources dedicated to reduce the disease burden, the decline in cases has stagnated in the past 2–5 years [2,3]; thus imposing a huge and cyclic economic burden upon the people in affected regions. In response, the United Nations has included malaria in the key milestones that must be tackled by 2030 under goal number three of the recently adopted Sustainable Development Goals (SDGs) [4]. This demands the immediate upscaling or modification of the available interventions and the development of new tools, including effective vaccines, for malaria control. However, the road toward malaria reduction, elimination, and eradication is not without daunting roadblocks, such as the emergence of insecticide resistant mosquitoes and drug resistant malaria parasites [5], failing global financial support, limited human resources in malaria endemic regions, difficulties in understanding naturally acquired immunity to malaria, and the lack of a licensed highly effective malaria vaccine [6].

The molecular mechanisms of malaria infection involve interactions between parasite and host proteins, which are partially understood and largely limited to in vitro studies [7]. Infection of the human host begins when the *Anopheles* mosquito, the definitive host, injects mature sporozoites during a blood meal, which rapidly migrate to and invade hepatocytes. Within these cells they develop and differentiate into thousands of merozoites, which are released into the bloodstream and bind to and invade erythrocytes [7–9]. Invasion and intracellular development within erythrocytes provides the parasite an immunologically protected niche for survival and propagation, but also initiates malaria symptoms and pathology [7–9]. Recent data suggest that a repertoire of parasite-encoded ligands, such as the merozoite surface protein (MSP) family, recognize and bind to the erythrocyte surface. The merozoite then orientates itself to bring its apical end into direct contact

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Abbreviations: WGCFS, Wheat germ cell-free system; SDGs, Sustainable development goals; GIA, Growth inhibition assay.

* Corresponding author.
E-mail address: takashima.eizo.mz@ehime-u.ac.jp (E. Takashima).

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with the erythrocyte membrane, followed by formation of a tight junction. Other proteins such as EBA175, reticulocyte binding protein homologue 5 (PfRh5), AMA1-RON, and SUB1 participate in interactions with host receptors to bring about commitment to tight junction formation and invasion [7–10]. Since the parasite is exposed to host immune responses, erythrocyte invasion and the responsible parasite-encoded proteins present an attractive target for malaria vaccine and drug development [9,11,12].

The mechanisms of immunological protection against malaria are not well understood. It is known that naturally acquired immunity against clinical disease develops following repeated infections [13–15]. Transfusion of immunoglobulins taken from immune individuals dramatically improve malaria symptoms, suggesting that humoral immunity is critical for protection against the disease [16–19]. The current understanding is that antibodies can mediate protection from malaria infection and clinical disease alone or they may require cooperation with host immune cells (reviewed in [20]). Specifically, the functional mechanisms of antibodies may include blocking sporozoite traversal through tissues and inhibiting hepatocyte invasion [21,22]. Antibodies can also protect against clinical malaria by interfering with parasite processes such as adhesion and erythrocyte invasion by merozoites, thus impeding the intra-erythrocytic cycle; and blocking infected erythrocytes from binding to host endothelial receptors and sequestering in deep tissues [20]. For example, growth-inhibitory antibodies against PfRh5, a PfRh family protein essential for erythrocyte invasion, have been associated with delayed time to onset and reduced risk of clinical malaria [23,24]. The antigen targets of the protective immunoglobulins deserve further investigation, since the observations suggest that it is possible to make an efficacious malaria vaccine.

2. Challenges in malaria vaccine studies

The determination of the *P. falciparum* genome in the 2000s [25] opened the "omics" era - genomics, proteomics, and transcriptomics - and brought renewed promise for malaria studies. However, little success has been realized and malaria vaccine discovery efforts have yielded but a handful of vaccine candidates [26]. The few antigens that have progressed to clinical trials have yielded low or moderate efficacies, as extensively summarized in [27–29]. The leading vaccine, *P. falciparum* circumsporozoite antigen derived RTS,S, is currently undergoing pilot implementation studies in African countries; albeit with moderate and short-lived efficacy [30,31]. One constraint to develop malaria vaccines with high efficacies is the lack of understanding of the mechanisms of naturally acquired immunity in residents of malaria endemic regions, and a lack of well-defined correlates of protection. Data from malaria field studies in both natural infection and vaccine induced immunity has pointed to an important role of specific antigenic targets in driving the cellular and humoral immunity mediating protection [32]. Vaccine candidates such as AMA-1, MSP1, and GLURP induce robust protective antibody responses against homologous parasites, but their development has stagnated due to genetic polymorphism and the lack of a protective effect against heterologous parasite strains (reviewed in [33]). Nonetheless, recent technological advances have greatly improved the prospects for designing an effective malaria vaccine, via a combination of high-throughput biological studies, improved statistical and computation capacities, and improved adjuvants for use in clinical trials.

3. Malaria vaccine antigen discovery technologies

Understanding the function of proteins predicted from the *Plasmodium* genome sequence, including those that are targets of protective immunity, has been protractedly hindered by the practical difficulties in producing *Plasmodium* proteins by heterologous expression systems in native or functionally active states [34,35]. Characteristics of *P. falciparum* proteins that negatively impinge on production include:

(i) *P. falciparum* genes have a remarkably low GC content (~ 20%), and have codons that are infrequently used by heterologous protein expression organisms [25,36].
(ii) Many *Plasmodium* proteins are unusually enriched in extensive and repetitive low-complexity sequences containing a limited repertoire of amino acids [37,38].
(iii) Most proteins are of high molecular weight (>60 kDa), possess disordered regions, and have basic pI (> 6) [25,35].
(iv) The presence of export motifs and/or signal peptide sequences can negatively impact recombinant protein expression [35,39].
(v) Extracellular proteins typically have multiple disulfide bonds to stabilize correct folding. Their globular protein domains, such as epidermal growth factor (EGF)-like motifs, are considered attractive candidates for antimalarial vaccines, but their disulfide-bonded structures make them difficult to express [40,41].

Nevertheless, in the last decade diverse approaches have been applied to express recombinant *Plasmodium* proteins, including both cell-based and cell-free technologies (Table 1). The major cell-based systems include expression platforms using bacterial [35,42,43]; yeast such as *Saccharomyces cerevisiae* [44,45] and *Pichia pastoris* [46,47]; *Dictyostelium discoideum* [48]; insect, e.g. *Spodoptera frugiperda* Sf9; [49] silkworm [50]; plants [51]; algae [52]; and mammalian cells [53,54]. Cell-free systems exploit the protein synthesis power of normal cells while eliminating many of the constraints of cell-based systems, and are derived from cells such as rabbit reticulocyte [55], Chinese hamster ovary [56], insect [57], HeLa [58], *Leishmania tarentolae* [59], tobacco plant [60], archaeal [61], and yeast [62]. Other cell-free systems include those derived from bacteria such as *Escherichia coli* [63], *Bacillus subtilis* [64], *Vibrio natriegens* [65], *Pseudomonas putida* [66], *Bacillus megaterium* [64], and *Streptomyces* [67]. The use of codon optimized artificial genes continues to contribute to improved protein synthesis in different systems [68,69]. We note that cell-free systems gained attention for their ability to rapidly produce proteins under controlled conditions [70]. Indeed, *E. coli*-derived expression systems have been a mainstay of cell-free protein expression largely due to their easy adaptability, low cost, rapid deployment, and high expression yields [71]. However, the systematic expression of soluble functional *P. falciparum* proteins remains

Table 1
Examples of protein expression systems.

		References
Cell-based protein expression systems	Mammalian cells	[53,54].
	Bacterial	[35,42,43]
	Algae	[52]
	Plants	[51]
	Dictyostelium discoideum	[48]
	Silkworm	[50]
	Insect cells, *Spodoptera frugiperda* Sf9	[49]
	Yeast	
	Saccharomyces cerevisiae	[44,45]
	Pichia pastoris	[46,47]
Cell-free protein expression systems	HeLa	[58]
	Rabbit reticulocyte	[55]
	Archaeal	[61]
	Yeast	[62]
	Chinese hamster ovary	[56]
	Insect cells	[57]
	Leishmania tarentolae	[59]
	Tobacco	[60]
	Bacteria	
	Escherichia coli	[63]
	Pseudomonas putida	[66]
	Bacillus subtilis	[64]
	Streptomyces	[67]
	Vibrio natriegens	[65]
	Bacillus megaterium	[64]

difficult, with success rates being as low as 6% [72,73]. Consequently, the functional characterization of extracellular parasite proteins has typically been restricted to smaller protein fragments, such as peptide arrays [74], that can be expressed rather than full-length proteins or entire ectodomains, which are more likely to be representative of the native proteins. These are major challenges considering that extensive efforts are needed to generate a functional malarial protein library [34,42].

To overcome the above limitations, the wheat germ cell-free system (WGCFS) offers a eukaryotic alternative to express plasmodial proteins with wide applications in malaria studies (Fig. 1) [34,75,76]. In our early experiments, Tsuboi et al. 2008 generated soluble *P. falciparum* proteins and demonstrated that WGCFS expressed proteins are greatly advantageous over *E. coli* based system-derived proteins in the pursuit of obtaining high yields of full-length proteins [34]. Furthermore, it was reported that WGCFS-produced malaria proteins have greater immunoreactivity to human immune sera, as opposed to identical proteins produced in *E. coli*-cell free systems [72]. WGCFS is capable of expressing large and complex proteins in their near native forms with minimal or no codon optimization requirements [75,76]. WGCFS yields proteins that can induce biologically functional antibodies in immunized animals, such as demonstrated by growth inhibition assays (GIA) [77]. The specificity of the recombinant proteins is suggested by their strong reactivity with sera obtained from individuals exposed to *P. falciparum* but not from malaria-naive donors [23,77,78]. These observations support that plasmodial protein domains are correctly folded as native proteins and require neither purification nor refolding. Recently, we and others have conducted several studies to validate these protein libraries in several immune epidemiology studies in Asia and Africa [23,78–80].

4. WGCFS and immunoscreening approaches

Immuno-epidemiology studies have contributed immensely to the identification or characterization of most of the current candidate antimalaria vaccines in the developmental pipeline. High-throughput immunoscreening to simultaneously investigate the interactions of proteins as immune correlates of protection and potential vaccine candidates has been a major strategy in malaria studies [23,42,53,81]. Some of the identified antigens include AMA1, PfRh5, CelTOS, MSP3, and GLURP [23,78,82]. Still, the identification of new targets will advance vaccine discovery through the prioritization of more immunogenic, multiple parasite stage expressed antigens not only for *P. falciparum*, but also for *P. vivax* and other malaria causing species.

Through collaborative efforts we have developed and optimized an innovative high-throughput WGCFS based antibody screening system [78,80,83]. This approach has prioritized several *P. falciparum* antigens for inclusion in the vaccine development pipeline following the immunoscreening of hundreds of parasite proteins expressed in multiple parasite stages. In a recent study for vaccine candidate discovery a total of 1827 recombinant proteins were drawn from different *P. falciparum* stages (sporozoites, merozoites, trophozoites, schizonts, and gametocytes) and expressed using WGCFS [78]. The proteins were used to probe individual serum samples obtained from residents of a malaria endemic region in Uganda. In the population 54% of the proteins were immunoreactive. Among them, 128 antigens induced antibody responses that significantly correlated with protective immunity. Antigen prioritization based on immunoscreening studies has traditionally focused on proteins putatively expressed on the surface of infected erythrocytes or infective stages (merozoites and sporozoites) by virtue of having a signal peptide (SP) and/or transmembrane domain (TM) [23,53,81]. As such, 53 antigens were selected as rational targets of a host protective immune response since they are predicted to have a SP and/or TM, or are confirmed to be localized on the parasite surface. In a similar approach, by targeting parasite protein families that are exported to the surface of infected red blood cells such as erythrocyte membrane protein 1 (PfEMP1), surface-associated interspersed gene family (SURFIN), repetitive interspersed family (RIFIN) proteins, and subtelomeric variable open reading frame (STEVOR) [84], we observed that more than 95% of the antigens were reactive with serum samples obtained from Uganda [85,86]. These studies clearly suggest that the repertoire of potentially protective antigens is wider than thought and offers multiple options for the identification of malaria vaccine candidates. One reassuring observation was that several of the selected antigens are currently under clinical or pre-clinical evaluation as vaccine antigens [78]. Similarly, WGCFS based surveys have contributed to immuno-epidemiological studies in *P. falciparum* infections in Papua New Guinea (PNG) [23], Mali [80], Tanzania [87], and in controlled human malaria infection investigations [88]; with additional antigens being selected for vaccine development or as correlates of protective immunity in malaria.

Numerous parasite proteins have been observed to reach the plasma membrane or extracellular space via unconventional routes [89–91]. Such atypical secreted proteins may escape conventional annotation since they lack SP but rather have cryptic motifs conferring trafficking to the cell surface. *Re*-analysis of available data collected in unbiased studies may ascertain additional protective antigens in malaria.

Multiple efforts have been ongoing over the last decade in several endemic regions to comprehensively evaluate naturally acquired antibody responses against *P. vivax* proteins. However, as with *P. falciparum* studies, difficulties in protein expression have been a major drawback. Recently it was reported in PNG that a greater breadth of antibody responses to multiple *P. vivax* antigens are present in asymptomatic individuals than in febrile patients [92]. No difference was seen in a study in similar groups in India [93] while the opposite was later reported in Thailand [94]. These disparities could be due to differences in the study designs in the different geographic regions or a result of distinct transmission intensities within the study sites. Alternatively, inconsistencies might be due to the use of different *P. vivax* protein microarrays encompassing separate sets of proteins, or the quality of the proteins used in the analyses. A direct comparison of naturally acquired antibody responses to different antigens is important to the identification of protective antigens, characterization of biomarkers of infection, and describing immunity in vivax malaria.

We have also established a high-throughput *P. vivax* antibody screening system using WGCFS technology. In its first use we determined antibody responses in sites in Thailand, Brazil, and PNG against 307 *P. vivax* proteins at the time of infection, and at several points in time in follow-up periods [79]. Most proteins in the panel were identified as immunoreactive in all sites. However, the antigens induced relatively short-lived antibody responses, with estimated half-lives of less than six months. Antibodies with relatively longer-lived responses were observed in PNG where individuals have had greater past exposure to *P. vivax*. A panel of 60 serological markers was identified capable of detecting exposure to *P. vivax* infections in the prior nine-month period. Subsequently, antibody responses to eight proteins with specificity for detecting recent infections were determined in independent studies conducted in Thailand, Brazil, and the Solomon Islands [95]. These biomarkers could help in the identification of possible hypnozoite carriers who should be targeted for treatment with liver-stage drugs such as primaquine. The antigens might also be used in screens to monitor future malaria elimination efforts through mass drug administration or other interventions.

WGCFS-based standardized high-throughput antibody detection methods offer unlimited options in future studies. To accelerate the identification of protective antibody targets, the methods can be applied widely as a common platform to facilitate immune evaluations between studies and across populations including malaria in children, in pregnancy, and in controlled human malaria infection studies. Moreover, the approaches produce large multi-dimensional data that can be computationally analyzed to generate testable hypotheses that may yield insights into the mechanisms underlying vaccine efficacy, and inform

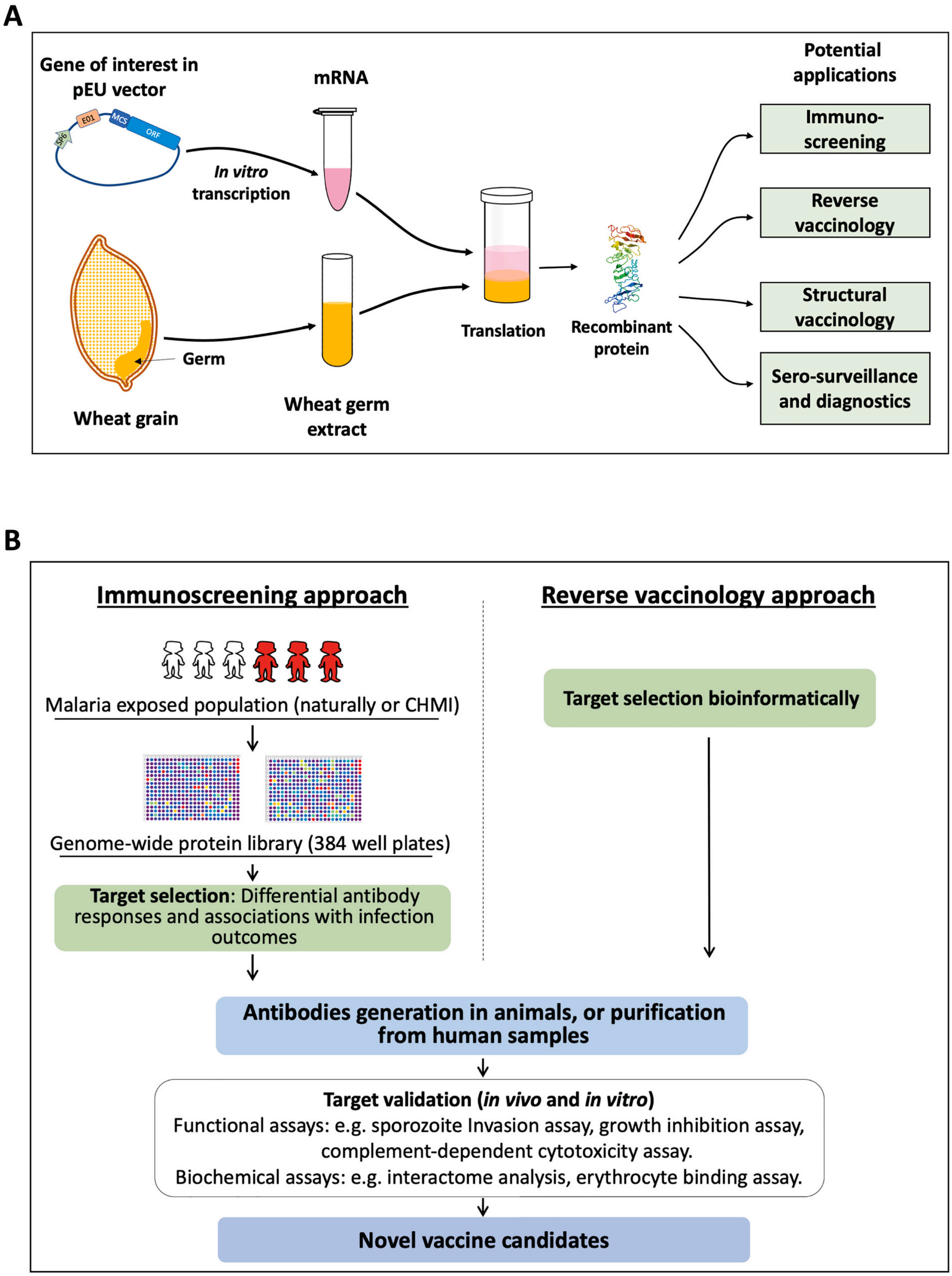

Fig. 1. Schematic illustration of wheat germ cell free protein expression system and its applications.

A.The DNA template for protein expression is prepared by cloning into pEU expression vectors or by direct PCR amplification followed by in vitro transcription to get mRNA. The mRNA is then mixed with wheat germ extract in the presence of blended amino acids, ATP, and GTP for in vitro translation. SP6 represents SP6 RNA polymerase promoter; E01, enhancer sequence; MCS, multiple cloning site; and ORF, open reading frame of the target gene.

B.Approaches for immunoscreening and reverse vaccinology-based vaccine target selection and validation using recombinant proteins synthesized using the WGCFS.

pathways on which vaccine strategies should focus.

5. WGCFS and reverse vaccinology

Reverse vaccinology and genome-based annotation hold significant promise in vaccine antigen discovery through the interrogation of comprehensive antigenic repertoires using libraries of select recombinantly expressed antigens. This is vital to deepening our understanding of the mechanisms of protection by naturally acquired and/or vaccine induced immunity; and will be needed to drive further studies to identify less immune but protective epitopes. Indeed, such in silico approaches have been used to select several novel vaccine antigens. For example, Pritam et al. systematically identified five potentially protective *P. falciparum* antigens with highly promiscuous T cell epitopes using bioinformatics tools; and prioritized the same for downstream evaluation [96]. Similarly, identification of conserved Pf48/45 and PfHAP2 genes as transmission blocking vaccine candidates was informed by reverse vaccinology analysis of *P. berghei* proteins [97–99]. The proteins are important targets due to their ability to diminish the fertility of male gametes.

In our recent study we annotated a selection of more than 300 proteins from the *P. falciparum* genome based upon the presence of SP and TM and aided by curations in malaria genome databases (PlasmoDB). The proteins were synthesized by WGCFS and used to raise antibodies in rabbits [100]. We observed that antibodies against a vaccine antigen, PfGAMA, co-immunoprecipitate with PfMSP10. Both PfGAMA and PfMSP10 co-localize on the surface of free merozoites and a direct interaction between the two was validated by surface plasmon resonance (SPR) [101]. Additionally, antibodies against PfMSP10 have growth inhibitory activity against cultured parasites, with the N-terminal region PfMSP10 R1 critical for interaction with its principal target, PfGAMA [101]. We propose PfMSP10 R1 as a candidate for further evaluation as an asexual-blood stage malaria vaccine antigen.

Similarly, we have observed that antibodies against the PfMSA180 C-terminal region, which contains an erythrocyte-binding domain, have GIA activity [102]. Further characterization by SPR showed that PfMSA180 specifically interacts with human erythrocyte integrin associated protein (CD47), suggesting that PfMSA180 has a role during merozoite invasion of erythrocytes. Naturally acquired PfMSA180-specific antibodies responses are associated with reduced risk to malaria in a Thai population [103]. Another protein of interest is a leading subunit vaccine candidate, PfRipr. PfRipr is highly conserved among African parasite isolates and forms a complex with CyRPA/Rh5-basigin which is suggested to play a central role in the sequential molecular events leading to merozoite invasion [104,105]. Through WGCFS synthesis of truncates of this large cysteine-rich molecule it has been demonstrated that antibodies against a region spanning C_{720}-D_{934}, which lies within the C-terminal EGF-like domains, potently inhibit merozoite invasion of erythrocytes [106]. The antibodies also strongly impede PfRipr interaction with Rh5 and SEMA7A, an erythrocyte-surface receptor, suggesting the inhibitory mode of action. PfRipr is a short and less complex molecule that is amicable to the good manufacturing practice (GMP) compatible baculovirus-based vaccine production system, and further development as a blood-stage malaria vaccine should be pursued.

Improvements in computing power now allow predictive algorithms to identify T and B cell epitopes as well as to accurately assess highly protective epitopes before recombinant protein production and antigen inclusion in vaccine development pipelines [107]. By utilizing WGCFS in conjunction with other reverse vaccinology approaches we may accelerate this process, especially for disease causing pathogens with complex genomes such as malaria parasites.

6. WGCFS and structural vaccinology

Structural vaccinology will accelerate studies into the structures of *P. falciparum* proteins through the identification of functional epitopes prioritized for further development [108,109]. Such functional epitopes can be tailored, either singly or as components of potent multivalent subunit vaccines, to circumvent some of the challenges in studies of malaria vaccines which include highly immunogenic but non-protective epitopes that the parasites might use to induce a “smoke-screen effect” to evade host immunity. Recently we observed that although antibody responses against full length PfMSP10 did not associate with clinical protection, antibodies against a short N-terminal region predicted clinical protection [101]. It should now be determined which linear or structural PfMSP10 N-terminal region epitopes induce protective immunity. Structural studies enabled Gangnard et al to identify a highly conserved interface between the DBL3X and DBL4ε domains that contributes significantly to the structuring of the functional VAR2CSA extracellular region [110]. This also holds true for the characterization of the three-dimensional structure of the invasion ligand cysteine-rich protective antigen (CyRPA) that was identified as containing a protective epitope which adopts a 6-bladed β-propeller structure similar to a sialidase fold but without enzymatic function [111]. Antibodies against CyRPA and its interacting partner PfRh5 have robust GIA activities [111].

Although protein crystallization is a strong approach for characterizing malaria parasite proteins, it is hampered by limitations in the synthesis of correctly folded target proteins produced in quantity and quality. An advantage of WGCFS is the linear scalability of the reaction volumes that allow small scale trials to determine recombinant protein suitability, followed by rapid scale up in production for high-resolution cryo-EM, X-ray crystallography structural studies, or isotope-labeled proteins for NMR structure analyses [112]. Using several target genes, Novicova and colleagues recently described WGCFS as a multiscale pipeline that allows quick screening for protein solubility, optimization for high yield expression, purification strategies suitable for high-resolution structural investigations, and feasibility of high-throughput screening, while avoiding potential toxicity issues [113]. We anticipate that soon malaria vaccines will incorporate structurally defined epitopes that elicit strong protective immune responses.

7. Future directions

A key strength of cell-free protein production systems is that they are amenable to direct manipulation at the transcription and translation steps. Customization is possible such as the expression of proteins with unnatural amino acids [114]; labeling with markers; and complex protein expression in lipid compartments such as liposomes [115], making it possible to study transmembrane proteins. However, some barriers must be overcome for WGCFS to be a complete system of choice. For example, the system lacks post translational glycosylation mechanisms for the production of glycoproteins. This drawback might be overcome by the recent development of a cell-free transcription-translation system enriched with glycosylation machinery which integrates protein biosynthesis with asparagine-linked protein glycosylation [116]. WGCFS could be heterologously enriched with functionally active WGCFS-produced oligosaccharyltransferases that catalyze *N*-linked or *O*-linked glycosyl transfer reactions, or combined with engineered glycan biosynthesis pathways [117].

It will be important to make WGCFS meet the regulatory requirements for vaccine manufacturing including GMP compliance for inclusion in the development of vaccines for preclinical testing in animal models and clinical studies in humans. This has been a major drawback considering that a good system requires substantial investment. GMP compliant systems have commercial goals such as high quality, high yields, and low costs; and WGCFS may offer the robustness needed to support these requirements.

The challenges in malaria vaccine development mirror those for other infectious diseases; such as the lack of a clear understanding of T and B cell immune responses to malaria, limited data on antigen(s) required for sterile protection, inadequate understanding on how to efficiently induce a long-lasting protective immune response, and how to identify the optimal means to harness innate immunity. However, new candidate vaccines may soon be available that are identified via the recent advent of high-throughput immunoscreening, reverse genomics approaches, lymphocyte repertoire sequencing [118], structural design of immunogens [119], and utilizing artificial intelligence (AI) [120,121]. Furthermore, with the explosion of next-generation vaccine development platforms fast-tracked largely by the urgent need for interventions against other emerging diseases such as the COVID-19 pandemic [122], we anticipate that adapting these technologies to malaria will increase the possibilities of designing and developing highly protective vaccines in the next decade.

8. Conclusion

The development of a highly efficacious malaria vaccine faces many challenges, including those associated with the identification of critical vaccine targets. The wheat germ cell-free expression systems will be an integral part of testing malaria vaccine-oriented hypotheses, such as identification and characterization of functional interactions between specific parasite proteins and/or host molecules. A deeper understanding of the major factors underlying the immunological and molecular mechanisms of malaria might provide a comprehensive approach to identifying novel and highly efficacious vaccines. Ultimately, these investigations will contribute to the development of second-generation malaria vaccines suitable for use in malaria-endemic areas, as envisioned under the malaria vaccine technology roadmap toward 2030.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

[1] WHO, World Malaria Report 2019, World Health Organization, Geneva, Switzerland, Geneva, Switzerland, 2019.
[2] WHO, World Malaria Report 2016, World Health Organization, Geneva, Switzerland, Geneva, Switzerland, 2016.
[3] WHO, World Malaria Report 2017, World Health Organization, Geneva, Switzerland, Geneva, Switzerland, 2017.
[4] WHO, Global Technical Strategy for Malaria 2016–2030, World Health Organization, Geneva, Switzerland, Geneva, Switzerland, 2015.
[5] K. Haldar, S. Bhattacharjee, I. Safeukui, Drug resistance in Plasmodium, Nat. Rev. Microbiol. 16 (2018) 156–170.
[6] P.L. Alonso, G. Brown, M. Arevalo-Herrera, F. Binka, C. Chitnis, F. Collins, O. K. Doumbo, B. Greenwood, B.F. Hall, M.M. Levine, K. Mendis, R.D. Newman, C. V. Plowe, M.H. Rodriguez, R. Sinden, L. Slutsker, M. Tanner, A research agenda to underpin malaria eradication, PLoS Med. 8 (2011), e1000406.
[7] G.E. Weiss, P.R. Gilson, T. Taechalertpaisarn, W.H. Tham, N.W. de Jong, K. L. Harvey, F.J. Fowkes, P.N. Barlow, J.C. Rayner, G.J. Wright, A.F. Cowman, B. S. Crabb, Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during Plasmodium falciparum invasion of erythrocytes, PLoS Pathog. 11 (2015), e1004670.
[8] P.R. Gilson, B.S. Crabb, Morphology and kinetics of the three distinct phases of red blood cell invasion by Plasmodium falciparum merozoites, Int. J. Parasitol. 39 (2009) 91–96.
[9] A.F. Cowman, C.J. Tonkin, W.H. Tham, M.T. Duraisingh, The molecular basis of erythrocyte invasion by malaria parasites, Cell Host Microbe 22 (2017) 232–245.
[10] P. Srinivasan, W.L. Beatty, A. Diouf, R. Herrera, X. Ambroggio, J.K. Moch, J. S. Tyler, D.L. Narum, S.K. Pierce, J.C. Boothroyd, J.D. Haynes, L.H. Miller, Binding of Plasmodium merozoite proteins RON2 and AMA1 triggers commitment to invasion, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13275–13280.
[11] A.F. Cowman, D. Berry, J. Baum, The cellular and molecular basis for malaria parasite invasion of the human red blood cell, J. Cell Biol. 198 (2012) 961–971.
[12] J.G. Beeson, D.R. Drew, M.J. Boyle, G. Feng, F.J. Fowkes, J.S. Richards, Merozoite surface proteins in red blood cell invasion, immunity and vaccines against malaria, FEMS Microbiol. Rev. 40 (2016) 343–372.
[13] S. Gupta, R.W. Snow, C.A. Donnelly, K. Marsh, C. Newbold, Immunity to non-cerebral severe malaria is acquired after one or two infections, Nat. Med. 5 (1999) 340–343.
[14] I. Kleinschmidt, B. Sharp, Patterns in age-specific malaria incidence in a population exposed to low levels of malaria transmission intensity, Tropical Med. Int. Health 6 (2001) 986–991.
[15] P. Druilhe, J.L. Perignon, Mechanisms of defense against P. falciparum asexual blood stages in humans, Immunol. Lett. 41 (1994) 115–120.
[16] I.A. McGregor, Studies in the acquisition of immunity of Plasmodium falciparum infections in Africa, Trans. R. Soc. Trop. Med. Hyg. 58 (1964) 80–92.
[17] D.S. Rowe, I.A. McGregor, S.J. Smith, P. Hall, K. Williams, Plasma immunoglobulin concentrations in a west African (Gambian) community and in a group of healthy British adults, Clin. Exp. Immunol. 3 (1968) 63–79.
[18] H. Bouharoun-Tayoun, P. Attanath, A. Sabchareon, T. Chongsuphajaisiddhi, P. Druilhe, Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes, J. Exp. Med. 172 (1990) 1633–1641.
[19] A. Sabchareon, T. Burnouf, D. Ouattara, P. Attanath, H. Bouharoun-Tayoun, P. Chantavanich, C. Foucault, T. Chongsuphajaisiddhi, P. Druilhe, Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria, Am. J. Trop. Med. Hyg. 45 (1991) 297–308.
[20] A. Teo, G. Feng, G.V. Brown, J.G. Beeson, S.J. Rogerson, Functional antibodies and protection against blood-stage malaria, Trends Parasitol. 32 (2016) 887–898.
[21] M.T. White, P. Bejon, A. Olotu, J.T. Griffin, K. Bojang, J. Lusingu, N. Salim, S. Abdulla, N. Otsyula, S.T. Agnandji, B. Lell, K.P. Asante, S. Owusu-Agyei, E. Mahama, T. Agbenyega, D. Ansong, J. Sacarlal, J.J. Aponte, A.C. Ghani, A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS,S malaria vaccine, BMC Med. 12 (2014) 117.
[22] L. Foquet, C.C. Hermsen, G.J. van Gemert, E. Van Braeckel, K.E. Weening, R. Sauerwein, P. Meuleman, G. Leroux-Roels, Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent Plasmodium falciparum infection, J. Clin. Invest. 124 (2014) 140–144.
[23] J.S. Richards, T.U. Arumugam, L. Reiling, J. Healer, A.N. Hodder, F.J. Fowkes, N. Cross, C. Langer, S. Takeo, A.D. Uboldi, J.K. Thompson, P.R. Gilson, R. L. Coppel, P.M. Siba, C.L. King, M. Torii, C.E. Chitnis, D.L. Narum, I. Mueller, B. S. Crabb, A.F. Cowman, T. Tsuboi, J.G. Beeson, Identification and prioritization of merozoite antigens as targets of protective human immunity to Plasmodium falciparum malaria for vaccine and biomarker development, J. Immunol. 191 (2013) 795–809.
[24] T.M. Tran, A. Ongoiba, J. Coursen, C. Crosnier, A. Diouf, C.Y. Huang, S. Li, S. Doumbo, D. Doumtabe, Y. Kone, A. Bathily, S. Dia, M. Niangaly, C. Dara, J. Sangala, L.H. Miller, O.K. Doumbo, K. Kayentao, C.A. Long, K. Miura, G. J. Wright, B. Traore, P.D. Crompton, Naturally acquired antibodies specific for Plasmodium falciparum reticulocyte-binding protein homologue 5 inhibit parasite growth and predict protection from malaria, J. Infect. Dis. 209 (2014) 789–798.
[25] M.J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R.W. Hyman, J. M. Carlton, A. Pain, K.E. Nelson, S. Bowman, I.T. Paulsen, K. James, J.A. Eisen, K. Rutherford, S.L. Salzberg, A. Craig, S. Kyes, M.S. Chan, V. Nene, S.J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A.B. Vaidya, D.M. Martin, A.H. Fairlamb, M.J. Fraunholz, D.S. Roos, S.A. Ralph, G.I. McFadden, L.M. Cummings, G.M. Subramanian, C. Mungall, J. C. Venter, D.J. Carucci, S.L. Hoffman, C. Newbold, R.W. Davis, C.M. Fraser, B. Barrell, Genome sequence of the human malaria parasite Plasmodium falciparum, Nature 419 (2002) 498–511.
[26] K. Miura, Progress and prospects for blood-stage malaria vaccines, Expert. Rev. Vaccines 15 (2016) 765–781.
[27] S.J. Draper, B.K. Sack, C.R. King, C.M. Nielsen, J.C. Rayner, M.K. Higgins, C. A. Long, R.A. Seder, Malaria vaccines: recent advances and new horizons, Cell Host Microbe 24 (2018) 43–56.
[28] K.L. Wilson, K.L. Flanagan, M.D. Prakash, M. Plebanski, Malaria vaccines in the eradication era: current status and future perspectives, Expert. Rev. Vaccines 18 (2019) 133–151.
[29] P.E. Duffy, Gorres J. Patrick, Malaria vaccines since 2000: progress, priorities, products, NPJ. Vaccines 5 (2020) 48.
[30] RTS SCTP, Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial, Lancet 386 (2015) 31–45.
[31] C.A. Dimala, B.T. Kika, B.M. Kadia, H. Blencowe, Current challenges and proposed solutions to the effective implementation of the RTS, S/AS01 malaria vaccine program in sub-Saharan Africa: A systematic review, PLoS One 13 (2018), e0209744.

[32] M.F. Good, L.H. Miller, Interpreting challenge data from early phase malaria blood stage vaccine trials, Expert. Rev. Vaccines 17 (2018) 189–196.

[33] D.R. Salamanca, M. Gomez, A. Camargo, et al., Plasmodium falciparum Blood Stage Antimalarial Vaccines: An Analysis of Ongoing Clinical Trials and New Perspectives Related to Synthetic Vaccines, Front. Microbiol. 10 (2019) 2712.

[34] T. Tsuboi, S. Takeo, H. Iriko, L. Jin, M. Tsuchimochi, S. Matsuda, E.T. Han, H. Otsuki, O. Kaneko, J. Sattabongkot, R. Udomsangpetch, T. Sawasaki, M. Torii, Y. Endo, Wheat germ cell-free system-based production of malaria proteins for discovery of novel vaccine candidates, Infect. Immun. 76 (2008) 1702–1708.

[35] L.M. Birkholtz, G. Blatch, T.L. Coetzer, H.C. Hoppe, E. Human, E.J. Morris, Z. Ngcete, L. Oldfield, R. Roth, A. Shonhai, L. Stephens, A.I. Louw, Heterologous expression of plasmodial proteins for structural studies and functional annotation, Malar. J. 7 (2008) 197.

[36] H. Musto, H. Romero, A. Zavala, K. Jabbari, G. Bernardi, Synonymous codon choices in the extremely GC-poor genome of Plasmodium falciparum: compositional constraints and translational selection, J. Mol. Evol. 49 (1999) 27–35.

[37] H.M. Davies, S.D. Nofal, E.J. McLaughlin, A.R. Osborne, Repetitive sequences in malaria parasite proteins, FEMS Microbiol. Rev. 41 (2017) 923–940.

[38] V. Muralidharan, D.E. Goldberg, Asparagine repeats in Plasmodium falciparum proteins: good for nothing? PLoS Pathog. 9 (2013), e1003488.

[39] J.C. Aguiar, J. LaBaer, P.L. Blair, V.Y. Shamailova, M. Koundinya, J.A. Russell, F. Huang, W. Mar, R.M. Anthony, A. Witney, S.R. Caruana, L. Brizuela, J. B. Sacci Jr., S.L. Hoffman, D.J. Carucci, High-throughput generation of P. falciparum functional molecules by recombinational cloning, Genome Res. 14 (2004) 2076–2082.

[40] A.W. Stowers, Y. Zhang, R.L. Shimp, D.C. Kaslow, Structural conformers produced during malaria vaccine production in yeast, Yeast 18 (2001) 137–150.

[41] G.J. Wright, Signal initiation in biological systems: the properties and detection of transient extracellular protein interactions, Mol. BioSyst. 5 (2009) 1405–1412.

[42] D.L. Doolan, Y. Mu, B. Unal, S. Sundaresh, S. Hirst, C. Valdez, A. Randall, D. Molina, X. Liang, D.A. Freilich, J.A. Oloo, P.L. Blair, J.C. Aguiar, P. Baldi, D. H. Davies, P.L. Felgner, Profiling humoral immune responses to P. falciparum infection with protein microarrays, Proteomics 8 (2008) 4680–4694.

[43] J.A. Fernandez-Robledo, G.R. Vasta, Production of recombinant proteins from protozoan parasites, Trends Parasitol. 26 (2010) 244–254.

[44] G. Hannig, S.C. Makrides, Strategies for optimizing heterologous protein expression in Escherichia coli, Trends Biotechnol. 16 (1998) 54–60.

[45] M. Jidenko, R.C. Nielsen, T.L. Sorensen, J.V. Moller, M. le Maire, P. Nissen, C. Jaxel, Crystallization of a mammalian membrane protein overexpressed in Saccharomyces cerevisiae, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11687–11691.

[46] J.L. Cereghino, J.M. Cregg, Heterologous protein expression in the methylotrophic yeast Pichia pastoris, FEMS Microbiol. Rev. 24 (2000) 45–66.

[47] J.F. Tschopp, P.F. Brust, J.M. Cregg, C.A. Stillman, T.R. Gingeras, Expression of the lacZ gene from two methanol-regulated promoters in Pichia pastoris, Nucleic Acids Res. 15 (1987) 3859–3876.

[48] M.X. van Bemmelen, C. Beghdadi-Rais, C. Desponds, E. Vargas, S. Herrera, C. D. Reymond, N. Fasel, Expression and one-step purification of Plasmodium proteins in dictyostelium, Mol. Biochem. Parasitol. 111 (2000) 377–390.

[49] J. Rydzak, R. Kaczmarek, M. Czerwinski, J. Lukasiewicz, J. Tyborowska, B. Szewczyk, E. Jaskiewicz, The baculovirus-expressed binding region of Plasmodium falciparum EBA-140 ligand and its glycophorin C binding specificity, PLoS One 10 (2015), e0115437.

[50] V.K. Deo, Y. Inagaki, E.H. Murhandarwati, W. Asmara, T. Miyazaki, T. Kato, E. Y. Park, Sero-diagnostic potential of Plasmodium falciparum recombinant merozoite surface protein (MSP)-3 expressed in silkworm, Parasitol. Int. 72 (2019) 101938.

[51] V. Beiss, H. Spiegel, A. Boes, M. Scheuermayer, A. Reimann, S. Schillberg, R. Fischer, Plant expression and characterization of the transmission-blocking vaccine candidate PfGAP50, BMC Biotechnol. 15 (2015) 108.

[52] J.A. Gregory, F. Li, L.M. Tomosada, C.J. Cox, A.B. Topol, J.M. Vinetz, S. Mayfield, Algae-produced Pfs25 elicits antibodies that inhibit malaria transmission, PLoS One 7 (2012), e37179.

[53] F.H. Osier, M.J. Mackinnon, C. Crosnier, G. Fegan, G. Kamuyu, M. Wanaguru, E. Ogada, B. McDade, J.C. Rayner, G.J. Wright, K. Marsh, New antigens for a multicomponent blood-stage malaria vaccine, Sci. Transl. Med. 6 (2014) (247ra102).

[54] K.M. VanBuskirk, E. Sevova, J.H. Adams, Conserved residues in the Plasmodium vivax Duffy-binding protein ligand domain are critical for erythrocyte receptor recognition, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15754–15759.

[55] M. Anastasina, I. Terenin, S.J. Butcher, D.E. Kainov, A technique to increase protein yield in a rabbit reticulocyte lysate translation system, Biotechniques 56 (2014) 36–39.

[56] A.K. Brodel, A. Sonnabend, S. Kubick, Cell-free protein expression based on extracts from CHO cells, Biotechnol. Bioeng. 111 (2014) 25–36.

[57] T. Ezure, T. Suzuki, S. Higashide, E. Shintani, K. Endo, S. Kobayashi, M. Shikata, M. Ito, K. Tanimizu, O. Nishimura, Cell-free protein synthesis system prepared from insect cells by freeze-thawing, Biotechnol. Prog. 22 (2006) 1570–1577.

[58] S. Mikami, M. Masutani, N. Sonenberg, S. Yokoyama, H. Imataka, An efficient mammalian cell-free translation system supplemented with translation factors, Protein Expr. Purif. 46 (2006) 348–357.

[59] S. Mureev, O. Kovtun, U.T. Nguyen, K. Alexandrov, Species-independent translational leaders facilitate cell-free expression, Nat. Biotechnol. 27 (2009) 747–752.

[60] M. Buntru, S. Vogel, K. Stoff, H. Spiegel, S. Schillberg, A versatile coupled cell-free transcription-translation system based on tobacco BY-2 cell lysates, Biotechnol. Bioeng. 112 (2015) 867–878.

[61] T. Endoh, T. Kanai, Y.T. Sato, D.V. Liu, K. Yoshikawa, H. Atomi, T. Imanaka, Cell-free protein synthesis at high temperatures using the lysate of a hyperthermophile, J. Biotechnol. 126 (2006) 186–195.

[62] R. Gan, M.C. Jewett, A combined cell-free transcription-translation system from Saccharomyces cerevisiae for rapid and robust protein synthe, Biotechnol. J. 9 (2014) 641–651.

[63] F. Caschera, V. Noireaux, Synthesis of 2.3 mg/ml of protein with an all Escherichia coli cell-free transcription-translation system, Biochimie 99 (2014) 162–168.

[64] R. Kelwick, A.J. Webb, J.T. MacDonald, P.S. Freemont, Development of a Bacillus subtilis cell-free transcription-translation system for prototyping regulatory elements, Metab. Eng. 38 (2016) 370–381.

[65] B.J. Des Soye, S.R. Davidson, M.T. Weinstock, D.G. Gibson, M.C. Jewett, Establishing a high-yielding cell-free protein synthesis platform derived from Vibrio natriegens, ACS Synth. Biol. 7 (2018) 2245–2255.

[66] T.B. Cook, J.M. Rand, W. Nurani, D.K. Courtney, S.A. Liu, B.F. Pfleger, Genetic tools for reliable gene expression and recombineering in Pseudomonas putida, J. Ind. Microbiol. Biotechnol. 45 (2018) 517–527.

[67] J. Li, H. Wang, Y.C. Kwon, M.C. Jewett, Establishing a high yielding streptomyces-based cell-free protein synthesis system, Biotechnol. Bioeng. 114 (2017) 1343–1353.

[68] T. Sugiyama, K. Suzue, M. Okamoto, J. Inselburg, K. Tai, T. Horii, Production of recombinant SERA proteins of Plasmodium falciparum in Escherichia coli by using synthetic genes, Vaccine 14 (1996) 1069–1076.

[69] K. Singh, M. Burkhardt, S. Nakuchima, R. Herrera, O. Muratova, A.G. Gittis, E. Kelnhofer, K. Reiter, M. Smelkinson, D. Veltri, B.J. Swihart, R. Shimp Jr., V. Nguyen, B. Zhang, N.J. MacDonald, P.E. Duffy, D.N. Garboczi, D.L. Narum, Structure and function of a malaria transmission blocking vaccine targeting Pfs230 and Pfs230-Pfs48/45 proteins, Commun. Biol. 3 (2020) 395.

[70] E.D. Carlson, R. Gan, C.E. Hodgman, M.C. Jewett, Cell-free protein synthesis: applications come of age, Biotechnol. Adv. 30 (2012) 1185–1194.

[71] T. Kigawa, T. Yabuki, N. Matsuda, T. Matsuda, R. Nakajima, A. Tanaka, S. Yokoyama, Preparation of Escherichia coli cell extract for highly productive cell-free protein expression, J. Struct. Funct. Genom. 5 (2004) 63–68.

[72] E. Rui, C. Fernandez-Becerra, S. Takeo, S. Sanz, M.V. Lacerda, T. Tsuboi, H.A. del Portillo, Plasmodium vivax: comparison of immunogenicity among proteins expressed in the cell-free systems of Escherichia coli and wheat germ by suspension array assays, Malar. J. 10 (2011) 192.

[73] C. Mehlin, E. Boni, F.S. Buckner, L. Engel, T. Feist, M.H. Gelb, L. Haji, D. Kim, C. Liu, N. Mueller, P.J. Myler, J.T. Reddy, J.N. Sampson, E. Subramanian, W. C. Van Voorhis, E. Worthey, F. Zucker, W.G. Hol, Heterologous expression of proteins from Plasmodium falciparum: results from 1000 genes, Mol. Biochem. Parasitol. 148 (2006) 144–160.

[74] D.H. Davies, X. Liang, J.E. Hernandez, A. Randall, S. Hirst, Y. Mu, K.M. Romero, T.T. Nguyen, M. Kalantari-Dehaghi, S. Crotty, P. Baldi, L.P. Villarreal, P. L. Felgner, Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 547–552.

[75] T.U. Arumugam, D. Ito, E. Takashima, M. Tachibana, T. Ishino, M. Torii, T. Tsuboi, Application of wheat germ cell-free protein expression system for novel malaria vaccine candidate discovery, Expert. Rev. Vaccines 13 (2014) 75–85.

[76] K. Takai, T. Sawasaki, Y. Endo, Practical cell-free protein synthesis system using purified wheat embryos, Nat. Protoc. 5 (2010) 227–238.

[77] T.U. Arumugam, S. Takeo, T. Yamasaki, A. Thonkukiatkul, K. Miura, H. Otsuki, H. Zhou, C.A. Long, J. Sattabongkot, J. Thompson, D.W. Wilson, J.G. Beeson, J. Healer, B.S. Crabb, A.F. Cowman, M. Torii, T. Tsuboi, Discovery of GAMA, a Plasmodium falciparum merozoite micronemal protein, as a novel blood-stage vaccine candidate antigen, Infect. Immun. 79 (2011) 4523–4532.

[78] B.N. Kanoi, E. Takashima, M. Morita, M.T. White, N.M. Palacpac, E.H. Ntege, B. Balikagala, A. Yeka, T.G. Egwang, T. Horii, T. Tsuboi, Antibody profiles to wheat germ cell-free system synthesized Plasmodium falciparum proteins correlate with protection from symptomatic malaria in Uganda, Vaccine 35 (2017) 873–881.

[79] R.J. Longley, M.T. White, E. Takashima, M. Morita, B.N. Kanoi, C.S.N. Li Wai Suen, I. Betuela, A. Kuehn, P. Sripoorote, C.T. Franca, P. Siba, L.J. Robinson, M. Lacerda, J. Sattabongkot, T. Tsuboi, I. Mueller, Naturally acquired antibody responses to more than 300 Plasmodium vivax proteins in three geographic regions, PLoS Negl. Trop. Dis. 11 (2017), e0005888.

[80] M. Morita, E. Takashima, D. Ito, K. Miura, A. Thongkukiatkul, A. Diouf, R. M. Fairhurst, M. Diakite, C.A. Long, M. Torii, T. Tsuboi, Immunoscreening of Plasmodium falciparum proteins expressed in a wheat germ cell-free system reveals a novel malaria vaccine candidate, Sci. Rep. 7 (2017) 46086.

[81] P.D. Crompton, M.A. Kayala, B. Traore, K. Kayentao, A. Ongoiba, G.E. Weiss, D. M. Molina, C.R. Burk, M. Waisberg, A. Jasinskas, X. Tan, S. Doumbo, D. Doumtabe, Y. Kone, D.L. Narum, X. Liang, O.K. Doumbo, L.H. Miller, D. L. Doolan, P. Baldi, P.L. Felgner, S.K. Pierce, A prospective analysis of the ab response to Plasmodium falciparum before and after a malaria season by protein microarray, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 6958–6963.

[82] D. Dodoo, A. Aikins, K.A. Kusi, H. Lamptey, E. Remarque, P. Milligan, S. Bosomprah, R. Chilengi, Y.D. Osei, B.D. Akanmori, M. Theisen, Cohort study of the association of antibody levels to AMA1, MSP119, MSP3 and GLURP with protection from clinical malaria in Ghanaian children, Malar. J. 7 (2008) 142.

[83] K. Matsuoka, H. Komori, M. Nose, Y. Endo, T. Sawasaki, Simple screening method for autoantigen proteins using the N-terminal biotinylated protein library produced by wheat cell-free synthesis, J. Proteome Res. 9 (2010) 4264–4273.
[84] J.A. Chan, F.J. Fowkes, J.G. Beeson, Surface antigens of Plasmodium falciparum-infected erythrocytes as immune targets and malaria vaccine candidates, Cell. Mol. Life Sci. 71 (2014) 3633–3657.
[85] B.N. Kanoi, H. Nagaoka, M. Morita, M.T. White, N.M.Q. Palacpac, E.H. Ntege, B. Balikagala, A. Yeka, T.G. Egwang, T. Horii, T. Tsuboi, E. Takashima, Comprehensive analysis of antibody responses to Plasmodium falciparum erythrocyte membrane protein 1 domains, Vaccine 36 (2018) 6826–6833.
[86] B.N. Kanoi, H. Nagaoka, M.T. White, M. Morita, N.M.Q. Palacpac, E.H. Ntege, B. Balikagala, A. Yeka, T.G. Egwang, T. Horii, T. Tsuboi, E. Takashima, Global repertoire of human antibodies against Plasmodium falciparum RIFINs, SURFINs, and STEVORs in a malaria exposed population, Front. Immunol. 11 (2020) 893.
[87] F. Saito, K. Hirayasu, T. Satoh, C.W. Wang, J. Lusingu, T. Arimori, K. Shida, N.M. Q. Palacpac, S. Itagaki, S. Iwanaga, E. Takashima, T. Tsuboi, M. Kohyama, T. Suenaga, M. Colonna, J. Takagi, T. Lavstsen, T. Horii, H. Arase, Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors, Nature 552 (2017) 101–105.
[88] J.C. Aguiar, J. Bolton, J. Wanga, J.B. Sacci, H. Iriko, J.K. Mazeika, E.T. Han, K. Limbach, N.B. Patterson, M. Sedegah, A.M. Cruz, T. Tsuboi, S.L. Hoffman, D. Carucci, M.R. Hollingdale, E.D. Villasante, T.L. Richie, Discovery of novel Plasmodium falciparum pre-Erythrocytic antigens for vaccine development, PLoS One 10 (2015), e0136109.
[89] J. Kim, H.Y. Gee, M.G. Lee, Unconventional protein secretion - new insights into the pathogenesis and therapeutic targets of human diseases, J. Cell Sci. 131 (2018).
[90] C. Moskes, P.A. Burghaus, B. Wernli, U. Sauder, M. Durrenberger, B. Kappes, Export of Plasmodium falciparum calcium-dependent protein kinase 1 to the parasitophorous vacuole is dependent on three N-terminal membrane anchor motifs, Mol. Microbiol. 54 (2004) 676–691.
[91] T. Thavayogarajah, P. Gangopadhyay, S. Rahlfs, K. Becker, K. Lingelbach, J. M. Przyborski, A.A. Holder, Alternative protein secretion in the malaria parasite Plasmodium falciparum, PLoS One 10 (2015), e0125191.
[92] O.C. Finney, S.A. Danziger, D.M. Molina, M. Vignali, A. Takagi, M. Ji, D. I. Stanisic, P.M. Siba, X. Liang, J.D. Aitchison, I. Mueller, M.J. Gardner, R. Wang, Predicting antidisease immunity using proteome arrays and sera from children naturally exposed to malaria, Mol. Cell. Proteomics 13 (2014) 2646–2660.
[93] S. Uplekar, P.N. Rao, L. Ramanathapuram, V. Awasthi, K. Verma, P. Sutton, S. Z. Ali, A. Patel, L.G. Sri, S. Ravishankaran, N. Desai, N. Tandel, S. Choubey, P. Barla, D. Kanagaraj, A. Eapen, K. Pradhan, R. Singh, A. Jain, P.L. Felgner, D. H. Davies, J.M. Carlton, J. Das, Characterizing antibody responses to Plasmodium vivax and Plasmodium falciparum antigens in India using genome-scale protein microarrays, PLoS Negl. Trop. Dis. 11 (2017), e0005323.
[94] E. Baum, J. Sattabongkot, J. Sirichaisinthop, K. Kiattibutr, A. Jain, O. Taghavian, M.C. Lee, D. Huw Davies, L. Cui, P.L. Felgner, G. Yan, Common asymptomatic and submicroscopic malaria infections in Western Thailand revealed in longitudinal molecular and serological studies: a challenge to malaria elimination, Malar. J. 15 (2016) 333.
[95] R.J. Longley, M.T. White, E. Takashima, J. Brewster, M. Morita, M. Harbers, T. Obadia, L.J. Robinson, F. Matsuura, Z.S.J. Liu, C.S.N. Li-Wai-Suen, W.H. Tham, J. Healer, C. Huon, C.E. Chitnis, W. Nguitragool, W. Monteiro, C. Proietti, D. L. Doolan, A.M. Siqueira, X.C. Ding, I.J. Gonzalez, J. Kazura, M. Lacerda, J. Sattabongkot, T. Tsuboi, I. Mueller, Development and validation of serological markers for detecting recent Plasmodium vivax infection, Nat. Med. 26 (2020) 741–749.
[96] M. Pritam, G. Singh, S. Swaroop, A.K. Singh, S.P. Singh, Exploitation of reverse vaccinology and immunoinformatics as promising platform for genome-wide screening of new effective vaccine candidates against Plasmodium falciparum, BMC Bioinforma. 19 (2019) 468.
[97] A.M. Blagborough, R.E. Sinden, Plasmodium berghei HAP2 induces strong malaria transmission-blocking immunity in vivo and in vitro, Vaccine 27 (2009) 5187–5194.
[98] M.R. van Dijk, C.J. Janse, J. Thompson, A.P. Waters, J.A. Braks, H.J. Dodemont, H.G. Stunnenberg, G.J. van Gemert, R.W. Sauerwein, W. Eling, A central role for P48/45 in malaria parasite male gamete fertility, Cell 104 (2001) 153–164.
[99] K.M. Merino, G.P. Bansal, N. Kumar, Reduced immunogenicity of Plasmodium falciparum gamete surface antigen (Pfs48/45) in mice after disruption of disulphide bonds - evaluating effect of interferon-gamma-inducible lysosomal thiol reductase, Immunology 148 (2016) 433–447.
[100] M. Morita, H. Nagaoka, E.H. Ntege, B.N. Kanoi, D. Ito, T. Nakata, J.W. Lee, K. Tokunaga, T. Iimura, M. Torii, T. Tsuboi, E. Takashima, PV1, a novel Plasmodium falciparum merozoite dense granule protein, interacts with exported protein in infected erythrocytes, Sci. Rep. 8 (2018) 3696.
[101] H. Nagaoka, B.N. Kanoi, K. Jinoka, M. Morita, T.U. Arumugam, N.M.Q. Palacpac, T.G. Egwang, T. Horii, T. Tsuboi, E. Takashima, The N-terminal region of Plasmodium falciparum MSP10 is a target of protective antibodies in malaria and is important for PfGAMA/PfMSP10 interaction, Front. Immunol. 10 (2019) 2669.
[102] H. Nagaoka, C. Sasaoka, T. Yuguchi, B.N. Kanoi, D. Ito, M. Morita, R. Udomsangpetch, J. Sattabongkot, T. Ishino, T. Tsuboi, E. Takashima, PfMSA180 is a novel Plasmodium falciparum vaccine antigen that interacts with human erythrocyte integrin associated protein (CD47), Sci. Rep. 9 (2019) 5923.
[103] H. Sakamoto, S. Takeo, E. Takashima, K. Miura, B.N. Kanoi, T. Kaneko, E.T. Han, M. Tachibana, K. Matsuoka, J. Sattabongkot, R. Udomsangpetch, T. Ishino, T. Tsuboi, Identification of target proteins of clinical immunity to Plasmodium falciparum in a region of low malaria transmission, Parasitol. Int. 67 (2018) 203–208.
[104] J.C. Volz, A. Yap, X. Sisquella, J.K. Thompson, N.T. Lim, L.W. Whitehead, L. Chen, M. Lampe, W.H. Tham, D. Wilson, T. Nebl, D. Marapana, T. Triglia, W. Wong, K. L. Rogers, A.F. Cowman, Essential role of the PfRh5/PfRipr/CyRPA complex during Plasmodium falciparum invasion of erythrocytes, Cell Host Microbe 20 (2016) 60–71.
[105] E.H. Ntege, N. Arisue, D. Ito, T. Hasegawa, N.M. Palacpac, T.G. Egwang, T. Horii, E. Takashima, T. Tsuboi, Identification of Plasmodium falciparum reticulocyte binding protein homologue 5-interacting protein, PfRipr, as a highly conserved blood-stage malaria vaccine candidate, Vaccine 34 (2016) 5612–5622.
[106] H. Nagaoka, B.N. Kanoi, E.H. Ntege, M. Aoki, A. Fukushima, T. Tsuboi, E. Takashima, Antibodies against a short region of PfRipr inhibit Plasmodium falciparum merozoite invasion and PfRipr interaction with Rh5 and SEMA7A, Sci. Rep. 10 (2020) 6573.
[107] E.S. Bergmann-Leitner, S. Chaudhury, N.J. Steers, M. Sabato, V. Delvecchio, A. S. Wallqvist, C.F. Ockenhouse, E. Angov, Computational and experimental validation of B and T-cell epitopes of the in vivo immune response to a novel malarial antigen, PLoS One 8 (2013), e71610.
[108] S. Hollingshead, I. Jongerius, R.M. Exley, S. Johnson, S.M. Lea, C.M. Tang, Structure-based design of chimeric antigens for multivalent protein vaccines, Nat. Commun. 9 (2018) 1051.
[109] D. Wang, S. Zhang, Y. Zou, W. Yu, Y. Jiang, Y. Zhan, N. Wang, Y. Dong, Y. Yang, Structure-based Design of Porcine Circovirus Type 2 chimeric VLPs (cVLPs) displays foreign peptides on the capsid surface, Front. Cell. Infect. Microbiol. 8 (2018) 232.
[110] S. Gangnard, A. Lewit-Bentley, S. Dechavanne, A. Srivastava, F. Amirat, G. A. Bentley, B. Gamain, Structure of the DBL3X-DBL4epsilon region of the VAR2CSA placental malaria vaccine candidate: insight into DBL domain interactions, Sci. Rep. 5 (2015) 14868.
[111] P. Favuzza, E. Guffart, M. Tamborrini, B. Scherer, A.M. Dreyer, A.C. Rufer, J. Erny, J. Hoernschemeyer, R. Thoma, G. Schmid, B. Gsell, A. Lamelas, J. Benz, C. Joseph, H. Matile, G. Pluschke, M.G. Rudolph, Structure of the malaria vaccine candidate antigen CyRPA and its complex with a parasite invasion inhibitory antibody, Elife 6 (2017).
[112] T. Kigawa, T. Yabuki, Y. Yoshida, M. Tsutsui, Y. Ito, T. Shibata, S. Yokoyama, Cell-free production and stable-isotope labeling of milligram quantities of proteins, FEBS Lett. 442 (1999) 15–19.
[113] I.V. Novikova, N. Sharma, T. Moser, R. Sontag, Y. Liu, M.J. Collazo, D. Cascio, T. Shokuhfar, H. Hellmann, M. Knoblauch, J.E. Evans, Protein structural biology using cell-free platform from wheat germ, Adv. Struct. Chem. Imag. 4 (2018) 13.
[114] K. Wals, H. Ovaa, Unnatural amino acid incorporation in E. coli: current and future applications in the design of therapeutic proteins, Front. Chem. 2 (2014) 15.
[115] H. Takeda, T. Ogasawara, T. Ozawa, A. Muraguchi, P.J. Jih, R. Morishita, M. Uchigashima, M. Watanabe, T. Fujimoto, T. Iwasaki, Y. Endo, T. Sawasaki, Production of monoclonal antibodies against GPCR using cell-free synthesized GPCR antigen and biotinylated liposome-based interaction assay, Sci. Rep. 5 (2015) 11333.
[116] T. Jaroentomeechai, J.C. Stark, A. Natarajan, C.J. Glasscock, L.E. Yates, K.J. Hsu, M. Mrksich, M.C. Jewett, M.P. DeLisa, Author correction: single-pot glycoprotein biosynthesis using a cell-free transcription-translation system enriched with glycosylation machinery, Nat. Commun. 9 (2018) 3396.
[117] J.H. Merritt, A.A. Ollis, A.C. Fisher, M.P. DeLisa, Glycans-by-design: engineering bacteria for the biosynthesis of complex glycans and glycoconjugates, Biotechnol. Bioeng. 110 (2013) 1550–1564.
[118] D. Hou, C. Chen, E.J. Seely, S. Chen, Y. Song, High-throughput sequencing-based immune repertoire study during infectious disease, Front. Immunol. 7 (2016) 336.
[119] L. Liljeroos, E. Malito, I. Ferlenghi, M.J. Bottomley, Structural and computational biology in the Design of Immunogenic Vaccine Antigens, J Immunol Res 2015 (2015) 156241.
[120] A.I. Heinson, Y. Gunawardana, B. Moesker, C.C. Hume, E. Vataga, Y. Hall, E. Stylianou, H. McShane, A. Williams, M. Niranjan, C.H. Woelk, Enhancing the biological relevance of machine learning classifiers for reverse vaccinology, Int. J. Mol. Sci. 18 (2017).
[121] P. Jabbari, N. Rezaei, Artificial intelligence and immunotherapy, Expert. Rev. Clin. Immunol. 15 (2019) 689–691.
[122] D. van Riel, E. de Wit, Next-generation vaccine platforms for COVID-19, Nat. Mater. 19 (2020) 810–812.