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Mini Review

Dissection of the *Plasmodium vivax* reticulocyte binding-like proteins (PvRBPs)

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ABSTRACT

Human malaria, which is caused by infection with *Plasmodium*, is a serious global public health problem. The erythrocytic stages are responsible for all of the symptoms and pathologies associated with malaria. Compared with *Plasmodium falciparum* merozoites infected human erythrocytes, *Plasmodium vivax* merozoites primarily invade reticulocytes. Due to the recent availability of the *P. vivax* genomic and transcriptomic datasets, the *P. vivax* reticulocyte binding-like proteins (PvRBPs) have been updated. However, the precise roles of PvRBPs remain largely unknown. Thus, here we discuss advances in our knowledge of the molecular interactions involved in erythrocyte invasion by *Plasmodium* merozoites, focusing particularly on PvRBP1 and PvRBP2. We also discuss potential PvRBP receptors during reticulocyte invasion.

1. Introduction

Human malaria is a serious global public health problem that is caused by infection with five species of the intracellular parasitic protozoan genus *Plasmodium*, particularly *Plasmodium falciparum* and *Plasmodium vivax*, which are transmitted by *Anopheles* mosquitoes. Although rarely fatal as compared with *P. falciparum*, *P. vivax* is often the dominant species and significantly contributes to morbidity in endemic areas outside of Africa [1–3].

Merozoite of *Plasmodium* spp. invades host erythrocytes via a multistep invasion process [4,5]. Unlike *P. falciparum*, which exploits multiple parasite receptors to invade erythrocytes, *P. vivax* invasion is restricted to reticulocytes (young erythrocytes) that are Duffy positive; Duffy-negative individuals are resistant to *P. vivax* invasion [6]. Unexpectedly, Duffy-negative blood is able to be invaded by vivax merozoites, indicating the existence of alternative pathways of *P. vivax* invasion [7,8]. However, the mechanisms underlying the molecular interactions between parasite ligands and host-cell receptors during reticulocyte invasion remain unclear. Comparion of *P. falciparum* reticulocyte binding-like proteins (PfRhs) [9–19], *P. vivax* reticulocyte binding-like proteins (PvRBPs) have not been thoroughly investigated [20–24].

Here, we summarize the molecular interactions between *Plasmodium* merozoites and erythrocytes. We discuss recent studies of PvRBPs and explore their potential receptors on human reticulocytes. Future perspectives are also discussed.

2. *Plasmodium* merozoite and erythrocyte invasion

Malaria parasites have a complex life cycle with two stages: one that occurs in vector mosquitoes (female) and the other that occurs in the liver and blood of the vertebrate host [25]. During the blood stage of the life cycle, the asexual parasites (merozoites) invade erythrocytes and produce symptomatic disease. The invasion process is complex and involves a multi-step sequence that comprises three phases [26,27] (Fig. 1). In phase I, the merozoite is released and attaches to the erythrocyte. Preliminary attachment likely involves chance contact with an erythrocyte surface molecule. An interaction between merozoite surface protein 1 (MSP1), which forming a complex with MSP6 and MSP7 [28], and Band 3 has been identified [29] (Fig. 2). During phase II, the merozoite reorients and attaches irreversibly to the erythrocyte through tight junction formation. These steps are highly dependent on specific molecular interactions between parasite ligands and erythrocyte receptors. Proteins on the merozoite surface and in the apical organelles, including microneme, dense granules, and rhoptry, mediate host cell recognition. However, these molecular interactions have not been completely defined. The key parasite ligands are secreted from micronemes and rhoptries and include apical membrane antigen 1 (AMA1) [30], RON complex [31,32], erythrocyte binding-like family (EBL) and reticulocyte binding-like family (RBL) [33–35] (Fig. 2). Additionally, receptors on the erythrocyte plasma membrane, including Glycophorin A and C (GPA and GPC) [36], Duffy-antigen receptor for chemokines (DARC) [36], Complement receptor 1 (CR1) [15], and Basigin (CD147) [17] have also been identified (Fig. 2). AMA1 interacts with RON2 directly, which serves as a self-receptor and forms a complex with RON4 and RON5 [37,38] (Fig. 2). In phase III, the merozoite enters the

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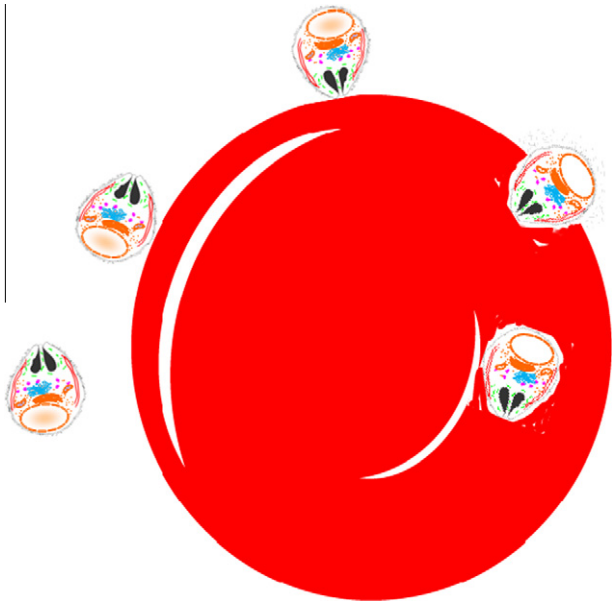

Fig. 1. A model of human erythrocyte invasion by *Plasmodium* merozoites. The invasion process is divided into three phases: phase I, merozoite release, initial recognition, and reversible attachment to the erythrocyte plasma membrane; phase II, reorientation and junction formation between the apical end of the merozoite and erythrocyte (irreversible attachment); phase III, merozoite entry into erythrocyte and parasitophorous vacuole (PV) formation.

erythrocyte and forms a parasitophorous vacuole (PV). This step is highly dynamic and rapid following apical attachment and tight junction formation, and involves a series of molecular events that guarantee erythrocyte entry and localization to a vacuole therein [27].

3. The *P. vivax* reticulocyte binding-like proteins (PvRBPs)

When the complete genome of the *P. vivax* Salvador I (Sal I) strain was sequenced, 11 *Pvrbp* genes were found, including two partial and nine full-length genes, of which two were pseudogenes [23] (Table 1). Thus, PvRBPs have potential for pre-clinical evaluation and are candidates for inclusion in a vivax malaria vaccine. This discovery aids understanding of the molecular mechanisms by which PvRBPs facilitate parasite attachment, and suggests the existence of an alternate vivax merozoite invasion pathway [39].

The erythrocytic *P. falciparum* transcriptome is a valuable resource for investigations of the *P. vivax* blood-stage transcriptome [40,41]. Several *Pvrbp* genes are highly expressed during the schizont stage, suggesting essential roles during the blood stage of these parasites [42]. These data also facilitate further study of the biology and genetic functions of this parasite and represent a vital resource for the development of methods for controlling vivax malaria.

Immunomics, which integrates genomics, proteomics, and molecular immunology, will facilitate identification of target antigens for inclusion in an effective malaria vaccine [43]. For high-throughput functional and structural analysis of *Plasmodium* proteins, the wheat germ cell-free protein synthesis system is a powerful tool and developed [44,45]. Following the approaches of the protein expression system and immunomics [43,46–48], we constructed fragments covering nine PvRBP members and screened sera from volunteers who were naturally exposed to vivax malaria by high-throughput manner. The immunoproteomics profiling results revealed that several PvRBP fragments were highly antigenic (unpublished data), suggesting that they may be candidates for inclusion in a vivax malaria vaccine.

4. PvRBP1 and PvRBP2

At the protein level, PvRBP1 and PvRBP2 of the Belem strain are 99% and 87% identical, respectively, to PvRBP1a and PvRBP2c of the Sal I strain [23]. PvRBP1a has a short deleted sequence (^{590}LQTVEKFYKEILDSKE^{605}) compared to PvRBP1 (Fig. 3). Although PvRBP2 and PvRBP2c share a high sequence identity, their structures are markedly different (Fig. 3).

PvRBP1 and PvRBP2 have been implicated in reticulocyte recognition and selection [20]. Native PvRBP1 is likely a transmembrane-anchored disulfide-linked protein, and along with PvRBP2, may function as an adhesive protein complex [4,20]. That RGD motif contained within PvRBP1 may provide a clue to the nature of its host cell receptor [20] (Fig. 3). Due to the large sizes of PvRBP1 and PvRBP2 (>300 kDa) (Table 1), functional domain identification is problematic. Thus, fragments covering the entire sequence were constructed and contained a region at the N-terminals of both proteins that exhibited reticulocyte-binding activity [49]. Furthermore, analyses of overlapping 15-mer PvRBP1 synthetic peptides indicated the existence of four reticulocyte-binding regions (RBR1-I to -IV), which showed high-affinity binding to reticulocytes [22] (Fig. 3). RBR1-III showed the most reticulocyte-binding sequences [22]. Nevertheless, synthetic peptides have limitations compared to native peptides, such as a lack of post-translational modifications and conformational motifs, which hamper further functional study. Thus, investigations of the functions of PvRBP1-I and the antigenicity profile of PvRBP1-III were conducted using recombinant proteins. PvRBP1-I binds to both erythrocytes and reticulocytes in a concentration-dependent manner [50]. PvRBP1-III was expressed successfully and showed antigenicity with *P. vivax*-infected human serum [51]. The structural features of PvRBP2 have also been reported. PvRBP2 and PvRBP1 share significant homology in a particular short region [21]. Compared with PvRBP1, which has a number of cysteine residues (17/2833, 0.60%), PvRBP2 has a C-terminus motif that is repeated seven times [21]. Upstream of this, a 24-amino acid sequence is repeated three times [21].

The function of a protein is usually related to its subcellular localization [52]. Therefore, subcellular localization by indirect immunofluorescence assay (IFA) or immunoelectron microscopy (IEM) is useful for inferring protein functions. PvRBP1 and PvRBP2 localize to the apical pole of merozoites [20]. However, their precise sub-localizations remain unknown. Elucidation of the localization of PvRBPs, particularly PvRBP1 and PvRBP2, would aid in understanding the roles of this family.

Continued investigations of the localization and genetic diversity of *Pvrbp* genes will aid in the further discovery and analysis of their function during reticulocyte invasion. These data will also facilitate development of an effective vivax malaria vaccine based on receptor-blockade strategies [21]. *Pvrbp1* and *Pvrbp2* polymorphisms were examined and the results suggested that the genetic diversity of the *Pvrbp* genes is generally limited [53]. However, the diversity of *Pvrbp2* was markedly higher than that of *Pvrbp1*, suggesting that different RBL domains may be evolving under different selection and functional pressures [53].

5. Other PvRBPs

Sequencing of the *P. vivax* genome eliminates the possibility that *P. vivax* has a relatively simple reticulocyte invasion mechanism. Instead, it likely possesses alternative invasion pathways, since differential expression of PfRHs is compactly linked to alternative invasion pathways [24]. The new members of the PvRBP family, particularly the PvRBP2 subgroup, could provide a diversity of *P. vivax* reticulocyte invasion mechanisms, comparable to that of *P. falciparum*.

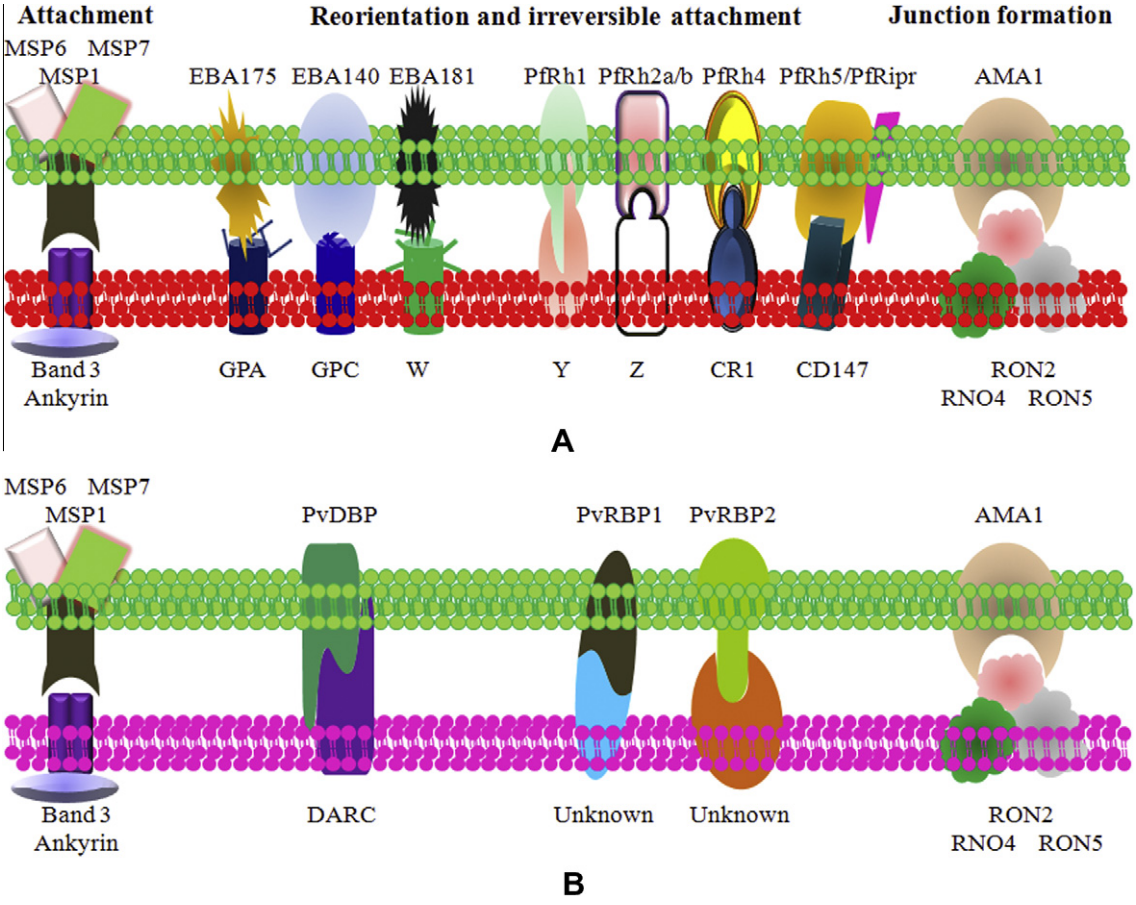

Fig. 2. Molecular mechanism of erythrocyte invasion by *Plasmodium* merozoites. (A) Molecular interaction between erythrocytes and the *P. falciparum* merozoite. The ligands of the *P. falciparum* merozoite include the MSP1 complex (MSP1, MSP6, and MSP7), EBL (EBA175, EBA140, and EBA181), PfRh (PfRh1, PfRh2a/b, PfRh4, and PfRh5 coupled with PfRipr) and the RON2 complex (RON2, RON4, and RON5). The receptors on the erythrocyte plasma membrane include band 3, GPA, GPC, CR1, and CD147. Abbreviations: GPA and GPC, glycophorin A and C; CR1, complement receptor 1; W, Y, and Z, unknown receptors. (B) Molecular interactions between reticulocytes and *P. vivax* merozoites. The ligands include the MSP1 complex, PvDBP, PvRBPs (PvRBP1 and PvRBP2), and RON2 complex of the *P. vivax* merozoite. The receptors on the reticulocyte plasma membrane include band 3 and several whose identities are unknown.

Table 1
General information of *Plasmodium vivax* reticulocyte binding-like proteins (PvRBPs).

Name	Gene ID	Strain	Chromosome No.	No. exons	CDS length	MW (kDa)	IP	Length	No. TM	SP	Ortholog group	References
PvRBP1	B42771/ Q00798[a]	Belem	7	Unknown	8502	303.3	5.57	2833	0	Yes		[20]
PvRBP1a	PVX_098585[b]	Sal I	7	3	8454	324.4	4.74	2817	0	Yes	OG5_130526	[23]
PvRBP1b	PVX_098582[b]	Sal I	7	1	7818	303.3	5.75	2605	0	Null	OG5_130526	[23]
PvRBP1-P[c]	PVX_125738[b]	Sal I	Not assigned	1	2361	90.9	4.99	786	1	Null	OG5_130526	[23]
PvRBP2	Q00799[b]	Belem	5	Unknown	8604	331.4	5.44	2867	1	Yes		[20]
PvRBP2a	PVX_121920[b]	Sal I	14	2	7464	286.7	7.26	2487	0	Yes	OG5_130526	[23]
PvRBP2b	PVX_094255[b]	Sal I	8	2	7994	308.3	5.63	2652	0	Null	OG5_130526	[23]
PvRBP2c	PVX_090325[b]	Sal I	5	1	8340	321.3	5.42	2779	1	Null	OG5_130526	[23]
PvRBP2-P1[c]	PVX_090330[b]	Sal I	5	2	1989	77.4	8.21	662	0	Null	OG5_179897	[23]
PvRBP2-P2[c]	PVX_101590[b]	Sal I	14	1	1926	74.1	9.35	641	0	Null	OG5_179897	[23]
PvRBP2hB[c]	PVX_116930[b]	Sal I	12	1	3958	124.0	9.57	1061	0	Null	OG5_167399	[23]
PvRBP2d[d]	PVX_101495[b]	Sal I	14	1	8495	308.4	10.34	2900	0	Null	Null	[23,24]
PvRBP3[d]	PVX_101585[b]	Sal I	14	1	8702	318.2	12.35	2831	1	Null	OG5_130526	[23,24]

Abbreviations: CDS length, coding sequence length; MW, molecular weight; Length, length of amino acid; IP, isoelectric point; SP, signal peptide; TM, transmembrane domain.
[a] GenBank accession No.
[b] PlasmoDB gene No.
[c] Partial fragments.
[d] Pseudogene.

Genetic diversity examinations of the newly discovered *Pvrbp* genes were evaluated [24]. Compared to the reference Sal-I strain, significant polymorphisms were identified in the *Pvrbp2a*, *Pvrbp2b*, *Pvrbp2d,* and *Pvrbp3* genes [24]. Although the polymorphism exhibited by the *Pvrbp2a* and *Pvrbp2b* genes was higher than that of *Pvrbp1* gene, it is far lower than that of *Pvrbp2* [24,53]. The

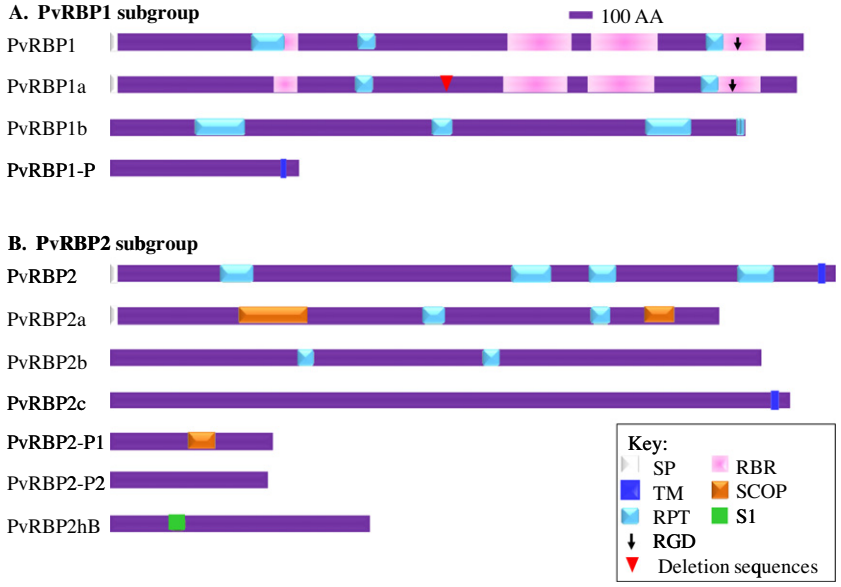

Fig. 3. PvRBP subgroups. (A) The PvRBP1 subgroup. Shown is the domain structure of the PvRBP1 subgroup that includes PvRBP1 (PvRBP1a), PvRBP1b, and PvRBP1-P. (B) The PvRBP2 subgroup. Shown is the domain structure of the PvRBP2 subgroup that includes PvRBP2 (PvRBP2c), PvRBP2a, PvRBP2b, PvRBP2-P1, PvRBP2-P2, and PvRBP2hB. Abbreviations: SP, signal peptide; TM, transmembrane domain; RPT, internal repeat region; RGD, Arg-Gly-Asp; RBR, reticulocyte binding region by peptides; SCOP, structural classification of proteins; S1, ribosomal protein S1-like RNA-binding domain.

Table 2
Summary of previously identified PfRhs and PvRBPs.

Organism	Ligand (Gene ID)	Receptor	Adhesion	Antibodies	Localization	References
P. vivax	RBP1(B42771/Q00798)	Unknown	Yes	Unknown	Apical end	[20]
	RBP2 (Q00799)	Unknown	Yes	Unknown	Apical end	[20]
P. falciparum	Rh1 (PFD0110w)	Y[a]	Yes	Block	Rhoptry neck	[9,12]
	Rh2a (PF13_0198)	Z[a]	Yes	Block	Rhoptry neck	[10,14,16,19]
	Rh2b (MAL13P1.176)	Z[a]	Yes	Block	Rhoptry neck	[10,16,18]
	Rh4 (PFD1150c)	CR1	Yes	Block	Rhoptry neck	[11,13,15]
	Rh5 (PFD1145c)	CD147	Yes	Block	Rhoptry neck	[17]

Abbreviations: CR1, Complement receptor 1; CD147, Basigin.
[a] Unknown receptors.

evolution of the PvRBP2 subgroup and their relatively high polymorphism rates suggest that these proteins are under immune selection and vital for parasite survival. In addition, highly conserved regions of *Pvrbp2a* and *Pvrbp2b* might be suitable targets for the development of vaccines against vivax malaria [24].

6. Human reticulocyte plasma membrane proteins as potential PvRBP receptors

Compared to PfRHs, for which several erythrocyte receptors have been identified [15,17], the reticulocyte receptors of PvRBPs remain largely unknown (Table 2 and Fig. 2). Reticulocytes are immature red blood cells (RBCs) and are present in the circulation at lower concentrations (typically ~1% of RBCs) as compared with mature or aged erythrocytes, which makes long-term *in vitro* culture of the *P. vivax* parasite difficult. Fortunately, a reliable *ex vivo* invasion assay that facilitates validation of the roles of PvRBPs during reticulocyte invasion is available [54].

Furthermore, identification and characterization of the highly abundant or unique reticulocyte plasma membrane proteins based on comparative proteomic analyses of erythrocytes and reticulocytes are necessary to identify PvRBP receptors. Recently, a comparison of the membrane proteome of murine reticulocytes with that of aged erythrocytes revealed several plasma membrane proteins that are candidate receptors for malaria parasite entry [55,56]. The human reticulocyte proteome remains unknown compared to that of human erythrocytes [57,58]. The reticulocyte transcriptome has been investigated; 20 highly represented genes were identified in cord- and adult-blood reticulocytes [59]. These data will facilitate investigation of the identities of reticulocyte PvRBP receptors.

7. Conclusions and perspectives

Binding of PvRBP1 and PvRBP2 binding to reticulocytes is independent of the Duffy antigen and suggests the existence of novel reticulocyte receptors for *P. vivax* merozoites. However, ligand-receptor interactions between merozoites and reticulocytes, particularly the PvRBPs and their receptors, are weak and transient, making dissection of the molecular mechanisms difficult. Thus, use of several powerful techniques [17,60,61] may be necessary to identify PvRBP receptors. Elucidation of the molecular mechanism underlying the interaction between PvRBPs and reticulocyte plasma-membrane proteins will assist selection of targets for rational vivax malaria vaccine and drug design.

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References

[1] K. Mendis, B.J. Sina, P. Marchesini, R. Carter, The neglected burden of *Plasmodium vivax* malaria, Am. J. Trop. Med. Hyg. 64 (2001) 97–106.

[2] S.I. Hay, C.A. Guerra, A.J. Tatem, A.M. Noor, R.W. Snow, The global distribution and population at risk of malaria: past, present, and future, Lancet Infect. Dis. 4 (2004) 327–336.

[3] R.N. Price, E. Tjitra, C.A. Guerra, S. Yeung, N.J. White, N.M. Anstey, Vivax malaria: neglected and not benign, Am. J. Trop. Med. Hyg. 77 (2007) 79–87.

[4] M.R. Galinski, J.W. Barnwell, *Plasmodium vivax*: merozoites, invasion of reticulocytes and considerations for malaria vaccine development, Parasitol. Today 12 (1996) 20–29.

[5] A.F. Cowman, B.S. Crabb, Invasion of red blood cells by malaria parasites, Cell 124 (2006) 755–766.

[6] D.M. Langhi Jr., J.O. Bordin, Duffy blood group and malaria, Hematology 11 (2006) 389–398.

[7] D. Menard, C. Barnadas, C. Bouchier, C. Henry-Halldin, L.R. Gray, A. Ratsimbasoa, V. Thonier, J.F. Carod, O. Domarle, Y. Colin, O. Bertrand, J. Picot, C.L. King, B.T. Grimberg, O. Mercereau-Puijalon, P.A. Zimmerman, *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people, Proc. Natl. Acad. Sci. USA 107 (2010) 5967–5971.

[8] N. Wurtz, K. Mint Lekweiry, H. Bogreau, B. Pradines, C. Rogier, A. Ould Mohamed Salem Boukhary, J.E. Hafid, M.S. Ould Ahmedou Salem, J.F. Trape, L.K. Basco, S. Briolant, Vivax malaria in Mauritania includes infection of a Duffy-negative individual, Malar. J. 10 (2011) 336.

[9] J.C. Rayner, E. Vargas-Serrato, C.S. Huber, M.R. Galinski, J.W. Barnwell, A *Plasmodium falciparum* homologue of *Plasmodium vivax* reticulocyte binding protein (PvRBP1) defines a trypsin-resistant erythrocyte invasion pathway, J. Exp. Med. 194 (2001) 1571–1581.

[10] T. Triglia, J. Thompson, S.R. Caruana, M. Delorenzi, T. Speed, A.F. Cowman, Identification of proteins from *Plasmodium falciparum* that are homologous to reticulocyte binding proteins in *Plasmodium vivax*, Infect. Immun. 69 (2001) 1084–1092.

[11] D. Gaur, S. Singh, L. Jiang, A. Diouf, L.H. Miller, Recombinant *Plasmodium falciparum* reticulocyte homology protein 4 binds to erythrocytes and blocks invasion, Proc. Natl. Acad. Sci. USA 104 (2007) 17789–17794.

[12] X. Gao, K.P. Yeo, S.S. Aw, C. Kuss, J.K. Iyer, S. Genesan, R. Rajamanonmani, J. Lescar, Z. Bozdech, P.R. Preiser, Antibodies targeting the PfRH1 binding domain inhibit invasion of *Plasmodium falciparum* merozoites, PLoS Pathog. 4 (2008) e1000104.

[13] W.H. Tham, D.W. Wilson, L. Reiling, L. Chen, J.G. Beeson, A.F. Cowman, Antibodies to reticulocyte binding protein-like homologue 4 inhibit invasion of *Plasmodium falciparum* into human erythrocytes, Infect. Immun. 77 (2009) 2427–2435.

[14] J.D. Dvorin, A.K. Bei, B.I. Coleman, M.T. Duraisingh, Functional diversification between two related *Plasmodium falciparum* merozoite invasion ligands is determined by changes in the cytoplasmic domain, Mol. Microbiol. 75 (2010) 990–1006.

[15] W.H. Tham, D.W. Wilson, S. Lopaticki, C.Q. Schmidt, P.B. Tetteh-Quarcoo, P.N. Barlow, D. Richard, J.E. Corbin, J.G. Beeson, A.F. Cowman, Complement receptor 1 is the host erythrocyte receptor for *Plasmodium falciparum* PfRh4 invasion ligand, Proc. Natl. Acad. Sci. USA 107 (2010) 17327–17332.

[16] T. Triglia, L. Chen, S. Lopaticki, C. Dekiwadia, D.T. Riglar, A.N. Hodder, S.A. Ralph, J. Baum, A.F. Cowman, *Plasmodium falciparum* merozoite invasion is inhibited by antibodies that target the PfRh2a and b binding domains, PLoS Pathog. 7 (2011).

[17] C. Crosnier, L.Y. Bustamante, S.J. Bartholdson, A.K. Bei, M. Theron, M. Uchikawa, S. Mboup, O. Ndir, D.P. Kwiatkowski, M.T. Duraisingh, J.C. Rayner, G.J. Wright, Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*, Nature 480 (2011) 534–537.

[18] T. Sahar, K.S. Reddy, M. Bharadwaj, A.K. Pandey, S. Singh, C.E. Chitnis, D. Gaur, *Plasmodium falciparum* reticulocyte binding-like homologue protein 2 (PfRH2) is a key adhesive molecule involved in erythrocyte invasion, PLoS One 6 (2011) e17102.

[19] K. Gunalan, X. Gao, K.J. Liew, P.R. Preiser, Differences in erythrocyte receptor specificity of different parts of the *Plasmodium falciparum* reticulocyte binding protein homologue 2a, Infect. Immun. 79 (2011) 3421–3430.

[20] M.R. Galinski, C.C. Medina, P. Ingravallo, J.W. Barnwell, A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites, Cell 69 (1992) 1213–1226.

[21] M.R. Galinski, M. Xu, J.W. Barnwell, *Plasmodium vivax* reticulocyte binding protein-2 (PvRBP-2) shares structural features with PvRBP-1 and the *Plasmodium yoelii* 235 kDa rhoptry protein family, Mol. Biochem. Parasitol. 108 (2000) 257–262.

[22] M. Urquiza, M.A. Patarroyo, V. Mari, M. Ocampo, J. Suarez, R. Lopez, A. Puentes, H. Curtidor, J. Garcia, L.E. Rodriuez, R. Vera, A. Torres, M. Laverde, A.P. Robles, M.E. Patarroyo, Identification and polymorphism of *Plasmodium vivax* RBP-1 peptides which bind specifically to reticulocytes, Peptides 23 (2002) 2265–2277.

[23] J.M. Carlton, J.H. Adams, J.C. Silva, S.L. Bidwell, H. Lorenzi, E. Caler, J. Crabtree, S.V. Angiuoli, E.F. Merino, P. Amedeo, Q. Cheng, R.M. Coulson, B.S. Crabb, H.A. Del Portillo, K. Essien, T.V. Feldblyum, C. Fernandez-Becerra, P.R. Gilson, A.H. Gueye, X. Guo, S. Kang'a, T.W. Kooij, M. Korsinczky, E.V. Meyer, V. Nene, I. Paulsen, O. White, S.A. Ralph, Q. Ren, T.J. Sargeant, S.L. Salzberg, C.J. Stoeckert, S.A. Sullivan, M.M. Yamamoto, S.L. Hoffman, J.R. Wortman, M.J. Gardner, M.R. Galinski, J.W. Barnwell, C.M. Fraser-Liggett, Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*, Nature 455 (2008) 757–763.

[24] V. Kosaisavee, U. Lek-Uthai, R. Suwanarusk, A.C. Gruner, B. Russell, F. Nosten, L. Renia, G. Snounou, Genetic diversity in new members of the reticulocyte binding protein family in Thai *Plasmodium vivax* isolates, PLoS One 7 (2012) e32105.

[25] H.E. Shortt, Life-cycle of the mammalian malaria parasite, Br. Med. Bull. 8 (1951) 7–9.

[26] M. Aikawa, L.H. Miller, J. Johnson, J. Rabbege, Erythrocyte entry by malarial parasites. A moving junction between erythrocyte and parasite, J. Cell Biol. 77 (1978) 72–82.

[27] T.J. Hadley, Invasion of erythrocytes by malaria parasites: a cellular and molecular overview, Annu. Rev. Microbiol. 40 (1986) 451–477.

[28] M. Kadekoppala, A.A. Holder, Merozoite surface proteins of the malaria parasite: the MSP1 complex and the MSP7 family, Int. J. Parasitol. 40 (2010) 1155–1161.

[29] V.K. Goel, X. Li, H. Chen, S.C. Liu, A.H. Chishti, S.S. Oh, Band 3 is a host receptor binding merozoite surface protein 1 during the *Plasmodium falciparum* invasion of erythrocytes, Proc. Natl. Acad. Sci. USA 100 (2003) 5164–5169.

[30] J.C. Pizarro, B. Vulliez-Le Normand, M.L. Chesne-Seck, C.R. Collins, C. Withers-Martinez, F. Hackett, M.J. Blackman, B.W. Faber, E.J. Remarque, C.H. Kocken, A.W. Thomas, G.A. Bentley, Crystal structure of the malaria vaccine candidate apical membrane antigen 1, Science 308 (2005) 408–411.

[31] J. Cao, O. Kaneko, A. Thongkukiatkul, M. Tachibana, H. Otsuki, Q. Gao, T. Tsuboi, M. Torii, Rhoptry neck protein RON2 forms a complex with microneme protein AMA1 in *Plasmodium falciparum* merozoites, Parasitol. Int. 58 (2009) 29–35.

[32] N.I. Proellocks, R.L. Coppel, K.L. Waller, Dissecting the apicomplexan rhoptry neck proteins, Trends Parasitol. 26 (2010) 297–304.

[33] J. Iyer, A.C. Gruner, L. Renia, G. Snounou, P.R. Preiser, Invasion of host cells by malaria parasites: a tale of two protein families, Mol. Microbiol. 65 (2007) 231–249.

[34] S. Lopaticki, A.G. Maier, J. Thompson, D.W. Wilson, W.H. Tham, T. Triglia, A. Gout, T.P. Speed, J.G. Beeson, J. Healer, A.F. Cowman, Reticulocyte and erythrocyte binding-like proteins function cooperatively in invasion of human erythrocytes by malaria parasites, Infect. Immun. 79 (2011) 1107–1117.

[35] W.H. Tham, J. Healer, A.F. Cowman, Erythrocyte and reticulocyte binding-like proteins of *Plasmodium falciparum*, Trends Parasitol. 28 (2012) 23–30.

[36] S.S. Oh, A.H. Chishti, Host receptors in malaria merozoite invasion, Curr. Top. Microbiol. Immunol. 295 (2005) 203–232.

[37] 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. USA 108 (2011) 13275–13280.

[38] M.L. Tonkin, M. Roques, M.H. Lamarque, M. Pugniere, D. Douguet, J. Crawford, M. Lebrun, M.J. Boulanger, Host cell invasion by apicomplexan parasites: insights from the co-structure of AMA1 with a RON2 peptide, Science 333 (2011) 463–467.

[39] B. Mons, Preferential invasion of malarial merozoites into young red blood cells, Blood cells 16 (1990) 299–312.

[40] Z. Bozdech, M. Llinas, B.L. Pulliam, E.D. Wong, J. Zhu, J.L. DeRisi, The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*, PLoS Biol. 1 (2003) E5.

[41] K.G. Le Roch, Y. Zhou, P.L. Blair, M. Grainger, J.K. Moch, J.D. Haynes, P. De La Vega, A.A. Holder, S. Batalov, D.J. Carucci, E.A. Winzeler, Discovery of gene function by expression profiling of the malaria parasite life cycle, Science 301 (2003) 1503–1508.

[42] Z. Bozdech, S. Mok, G. Hu, M. Imwong, A. Jaidee, B. Russell, H. Ginsburg, F. Nosten, N.P. Day, N.J. White, J.M. Carlton, P.R. Preiser, The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites, Proc. Natl. Acad. Sci. USA 105 (2008) 16290–16295.

[43] D.L. Doolan, *Plasmodium* immunomics, Int. J. Parasitol. 41 (2011) 3–20.

[44] T. Tsuboi, S. Takeo, T.U. Arumugam, H. Otsuki, M. Torii, The wheat germ cell-free protein synthesis system: a key tool for novel malaria vaccine candidate discovery, Acta Trop. 114 (2010) 171–176.

[45] M. Madono, T. Sawasaki, R. Morishita, Y. Endo, Wheat germ cell-free protein production system for post-genomic research, N. Biotechnol. 28 (2011) 211–217.

[46] J.H. Chen, J.W. Jung, Y. Wang, K.S. Ha, F. Lu, C.S. Lim, S. Takeo, T. Tsuboi, E.T. Han, Immunoproteomics profiling of blood stage *Plasmodium vivax* infection by high-throughput screening assays, J. Proteome Res. 9 (2010) 6479–6489.

[47] J.H. Chen, Y. Wang, K.S. Ha, F. Lu, I.B. Suh, C.S. Lim, J.H. Park, S. Takeo, T. Tsuboi, E.T. Han, Measurement of naturally acquired humoral immune responses against the C-terminal region of the *Plasmodium vivax* MSP1 protein using protein arrays, Parasitol. Res. 109 (2011) 1259–1266.

[48] J. Li, D. Ito, J.H. Chen, F. Lu, Y. Cheng, B. Wang, K.S. Ha, J. Cao, M. Torii, J. Sattabongkot, T. Tsuboi, E.T. Han, Pv12, a 6-Cys antigen of *Plasmodium vivax*, is localized to the merozoite rhoptry, Parasitol. Int. 61 (2012) 443–449.

[49] M.R. Galinski, J.W. Barnwell, *Plasmodium vivax*: who cares?, Malar J. 7 (Suppl 1) (2008) S9.

[50] E.M. Cantor, T.B. Lombo, A. Cepeda, A.M. Espinosa, C.A. Barrero, F. Guzman, M.L. Gunturiz, M. Urquiza, M. Ocampo, M.E. Patarroyo, M.A. Patarroyo, *Plasmodium*

vivax: functional analysis of a highly conserved PvRBP-1 protein region, Mol. Biochem. Parasitol. 117 (2001) 229–234.
[51] J. Rojas Caraballo, G. Delgado, R. Rodriguez, M.A. Patarroyo, The antigenicity of a *Plasmodium vivax* reticulocyte binding protein-1 (PvRBP1) recombinant fragment in humans and its immunogenicity and protection studies in Aotus monkeys, Vaccine 25 (2007) 3713–3721.
[52] C.S. Yu, Y.C. Chen, C.H. Lu, J.K. Hwang, Prediction of protein subcellular localization, Proteins 64 (2006) 643–651.
[53] J.C. Rayner, T.M. Tran, V. Corredor, C.S. Huber, J.W. Barnwell, M.R. Galinski, Dramatic difference in diversity between *Plasmodium falciparum* and *Plasmodium vivax* reticulocyte binding-like genes, Am. J. Trop. Med. Hyg. 72 (2005) 666–674.
[54] B. Russell, R. Suwanarusk, C. Borlon, F.T. Costa, C.S. Chu, M.J. Rijken, K. Sriprawat, L. Warter, E.G. Koh, B. Malleret, Y. Colin, O. Bertrand, J.H. Adams, U. D'Alessandro, G. Snounou, F. Nosten, L. Renia, A reliable ex vivo invasion assay of human reticulocytes by *Plasmodium vivax*, Blood 118 (2011) e74–81.
[55] J.E. Prenni, A.C. Avery, C.S. Olver, Proteomics: a review and an example using the reticulocyte membrane proteome, Vet. Clin. Pathol. 36 (2007) 13–24.
[56] J.E. Prenni, M. Vidal, C.S. Olver, Preliminary characterization of the murine membrane reticulocyte proteome, Blood Cells Mol. Dis. (2012).
[57] D.G. Kakhniashvili, L.A. Bulla Jr., S.R. Goodman, The human erythrocyte proteome: analysis by ion trap mass spectrometry, Mol. Cell. Proteomics 3 (2004) 501–509.
[58] E.M. Pasini, M. Kirkegaard, P. Mortensen, H.U. Lutz, A.W. Thomas, M. Mann, In-depth analysis of the membrane and cytosolic proteome of red blood cells, Blood 108 (2006) 791–801.
[59] S.H. Goh, M. Josleyn, Y.T. Lee, R.L. Danner, R.B. Gherman, M.C. Cam, J.L. Miller, The human reticulocyte transcriptome, Physiol. Genomics 30 (2007) 172–178.
[60] G. Pichler, A. Jack, P. Wolf, S.B. Hake, Versatile toolbox for high throughput biochemical and functional studies with fluorescent fusion proteins, PLoS One 7 (2012) e36967.
[61] A. Jain, R.J. Liu, B. Ramani, E. Arauz, Y. Ishitsuka, K. Ragunathan, J. Park, J. Chen, Y.K. Xiang, T. Ha, Probing cellular protein complexes using single-molecule pull-down, Nature 473 (2011) 484–488.