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International Journal for Parasitology 34 (2004) 1441–1450
www.parasitology-online.com

Invited review

A proteomic analysis of malaria biology: integration of old literature and new technologies

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Received 5 June 2004; received in revised form 20 September 2004; accepted 1 October 2004

Abstract

The genomic revolution has brought a new vitality into research on *Plasmodium*, its insect and vertebrate hosts. At the cellular level nowhere is the impact greater than in the analysis of protein expression and the 'assembly' of the supramolecular machines that together comprise the functional cell. The repetitive phases of invasion and replication that typify the malaria life cycle, together with the unique phase of sexual differentiation provide a powerful platform on which to investigate the 'molecular machines' that underpin parasite strategy and stage-specific functions. This approach is illustrated here in an analysis of the ookinete of *Plasmodium berghei.* Such analyses are useful only if conducted with a secure understanding of parasite biology. The importance of carefully searching the older literature to reach this understanding cannot be over-emphasised. When viewed together, the old and new data can give rapid and penetrating insights into what some might now term the 'Systems-Biology' of *Plasmodium.*

Keywords: *Plasmodium*; Mosquito; Infection; Ookinete; Pellicle; Proteome

1. Introduction

The plethora of new research tools developed to study malaria biology, in combination with the global technologies of genomics, transcriptomics and proteomics, has transformed our understanding of *Plasmodium* at the molecular level. The key to future progress is to use these powerful technologies to address relevant biological questions. Whereas much of the molecular data is readily available through electronic media, the vast and detailed literature on malaria biology predates electronic archiving. Thus to make rapid progress with our new capabilities, and to avoid wasteful rediscovery of well-established biological principles it is essential to integrate the old and the new literature. Here I attempt to illustrate this concept with the application of a proteomic analysis of *Plasmodium berghei* to further our understanding of the biology of infection of the mosquito vector.

I believe now, more than at any time in the past it is necessary to emphasise to those planning malaria control, and those researching toward that objective our best opportunities for success in endemic areas once again lie in the control the parasite as it passes through the mosquito. Why do I believe this? My arguments are many-fold. Malaria distribution in endemic areas is largely defined by the distribution of the vector (Hay et al., 2002). Past experience of malaria control programmes has proved that a reduction in the vector population is an effective practical measure to limit the spread of disease (Bruce-Chwatt, 1985). In the field, the average parasite burden in an infected mosquito is only five to 50 cells (Sattabongkot et al., 1991); therefore the proportionate impact of intervention on the parasite population can be high. When considering the efficacy of any vaccine, the duration of exposure of the target cell to the immune system is a key variable (Anderson et al., 1989), in the midgut of the mosquito the sexual and ookinete stages are exposed to immune attack for 24 h, this compares with a few seconds in the case of the merozoite. Surface molecules expressed by the parasite de novo in the mosquito (e.g. P25, P28 CTRP, chitinase) are

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0020-7519/$30.00
doi:10.1016/j.ijpara.2004.10.005

less polymorphic than many of the comparable target proteins in the blood stage parasite, thus offering (relatively) stable targets for immune or other attack. Finally, the current repertoire of transmission-blocking vaccines targeting the ookinete surface (e.g. Pfs25/Pvs25) can be very effective (Hisaeda and Yasutomo, 2002). The recent efforts in our laboratory have therefore focussed on understanding the molecular cell biology of the process of infection of the mosquito vector, and, in particular, the biology of the ookinete.

2. Infection of the mosquito

When reviewing the literature on the infection of mosquito species by *Plasmodium* the extensive early work on *Plasmodium gallinaceum* (Table 1) indicated that the parasite could establish an oocyst infection in almost all mosquito genera tested, but that in *Culex* no salivary gland infections were seen. From this we conclude either: (i) the *P. gallinaceum* ookinete recognised different receptors in each mosquito species, or all the mosquitoes tested expressed a common receptor or (ii) a restricted repertoire of receptors were recognised by the sporozoite on the salivary gland basal lamina or basement membrane. In marked contrast, a similar review of the literature on *P. berghei* (Table 2) shows the ookinete transformed into an oocyst in many (but not all) anophelines, but it failed to produce oocysts in any culicine mosquitoes. By employing recent advances in the ability to examine gametogenesis and ookinete development in vitro as well as to investigate infected mosquitoes ex vivo, Alavi et al. (2003) extended the early infection studies and measured the losses incurred as the parasite passes through the insect midgut. Using *P. berghei* and *P. gallinaceum* to infect *Anopheles gambiae*, *Anopheles stephensi* and *Aedes aegypti* (Fig. 1), the events of gametogenesis and ookinete development were examined in particular detail. The key conclusions were that in susceptible mosquitoes losses in the midgut are significant (2–3 logs) and occur equally during transformation of the gametocyte into the ookinete, and following ookinete formation (i.e. during invasion of peritrophic matrix (PTM), midgut epithelium (ME) and interaction with the midgut basal lamina). Interestingly, they confirmed that *P. gallinaceum* invaded the ME of both culicines and anophelines and demonstrated that the cultured ookinete of *P. berghei* only invaded the *Anopheles* ME. Perhaps the most surprising observation was the inefficiency with which *P. berghei* gametocytes develop into ookinetes in the *Aedes* midgut. It was concluded this was due in part to the low xanthurenic acid (XA) production by *Aedes* combined with the high requirement of *P. berghei* for XA which, in combination, resulted in lower exflagellation (Arai et al., 2001), and in part to the destruction of the macrogametes and zygotes in the bloodmeal following fertilization. These data raise interesting, and unanswered, questions. Which of the numerous physiological differences in bloodmeal digestion between *Aedes* and *Anopheles* caused this destruction, and could these differences underpin the discovery of new inhibitors to integrate into transgenic refractory mosquitoes?

Following invasion of the ME by the ookinete, losses within the midgut epithelium were of similar magnitude in all susceptible parasite/vector combinations examined. These losses can be significantly greater in genetically selected refractory lines of mosquito (Vernick et al., 1995; Collins et al., 1986; Blandin et al., 2004; Osta et al., 2004). These refractory lines are known to lyse or melanise the ookinete, clearly emphasising the importance of the epithelium as a barrier to infection. The elegant studies of Blandin et al. (2004) and Osta et al. (2004) have shown conclusively that mosquito thioester-, or leucine-rich repeat-proteins present significant immune defences against the invading intracellular ookinete. It remains to be determined which ookinete surface proteins are recognised by these various arms of the mosquitoes' immune system.

3. Gametogenesis and signalling pathways

Following the discoveries that the mosquito waste product XA together with a fall in the temperature of 5 °C are both required to induce gametogenesis in vivo (Billker et al., 1998, 2000), further studies have shown that the probable signalling pathways involved include phospholipase C-mediated conversion of inositol biphosphate to triphosphate (Ogwang et al., 1993). This results in calcium release from cytoplasmic stores such as the endoplasmic reticulum (Kawamoto et al., 1990; Billker et al., 2004) that triggers (i) the redistribution of parasite proteins (e.g. Pf155/RESA (Quakyi et al., 1989)) into the parasitophorous vacuole; (ii) the expression of translationally repressed messenger RNAs such as those encoding P25 and P28 (Paton et al., 1993; Hall et al., 2004); and (iii) the activation of CDPK4. Billker et al. (2004) have demonstrated that CDPK4 in turn regulates both the three rounds of DNA replication; the polymerisation of microtubules forming the mitotic apparatus, and the flagellar axonemes previously described by Sinden et al. (1976). Clearly this parasite-specific signalling pathway is an attractive focus for the development of novel transmission-blocking drugs. Further, the observation of Alavi et al. (2003) that in *A. aegypti, P. berghei* is induced to initiate gametogenesis, but then fails to complete the process suggests factors in the bloodmeal inhibit either the downstream signalling pathway, and/or the ensuing morphological differentiation. It is important to know what these inhibitors and processes are because they could represent novel drug targets.

Table 1
Reported development of *Plasmodium gallinaceum* in different mosquito species

Mosquito		Exflagellation	Ookinete	Midgut invasion	Oocyst	Sporozoite formation	Salivary gland infection	Transmitted by bite
Genus	Species							
Aedes (*Stegomyia*)	*aegypti*[a,b,c,d,e,f,g]	+	+	+	+	+	+	+
	albopictus[b,h,c,i,j,k]		+		+	+	+	+
	pseudalbopictus[e]				+	+		
	scutellaris[e]				+	+		
	unilineatus[e]				+	+		
	vittatus[d,e]				+	+		
(*Aedimorphus*)	*jamesi*[d,j]				+			
	stokesi[l]				+	+		
	vexans[m,j]				+			
(*Finlaya*)	*albolatoralis*[j]				+			
	atropalpus[n]				+	+		+
	chrysolineatus[e]				+	+		
	geniculatus[o]				+	+		+
	japonicus[k]				+	+		+
	pallirostris[e]				+	+		+
	pseudotaeniatus[d,e]				+	+		
	togo[i,k]				+	+		+
	triseriatus[m]				+	+		
(*Ochlerotatus*)	*campestris*[m]				+	+		+
	canadensis[p]				+			
	cantator[m]				+	+		+
	lepidus[q]				+	+		+
	stimulans[m]				+	+		+
	trivittatus[m]				+			
Culiseta	*inornata*[m]				+			
Mansonia	*perturbans*[m]				+			
Armigeres	*annulipalpis*[j]				+			
	aureolineatus[e]				+	+		
	kuchingensis[e]				+	+		
	magnus[j]				+			
	obturbans[d,j,e]		+		+	+		
	subalbatus[k]				+	+		+
Culex (*Culex*)	*bitaeniorhynchus*[e,r,k]				−	−		−
	mimeticus[j,e]				−			
	pipiens[b,m]				−			−
	pipienspallens[k]				−	−		−
	quinquefasciatus[b,j,e,s]				−	+		−
	salinarius[m]				+			
	tarsalis[c]				+			
	tritaeniorhynchus[k]				−	−		−
(*Lutzia*)	*raptor*[j]				−			
(*Neoculex*)	*hayashi*[k]				−	−		−
Anopheles	*freeborni*[t,u]		+	+	+	+	Few	+
(*Anopheles*)	*gambiae*[v,g]	+	+	+	+	+	+	+
	gambiaeG3[v,k]				+		−	
	hyrcanus[j]				−			
	hyrcanus sinensis[k]				−	−		−
	quadrimaculatus[t,w,p]*		+	+	+	+black spores	Few	+
(*Nysorrhynchus*)	*Albimanus*[u]				+			−

(*continued on next page*)

Table 1 (*continued*)

Mosquito		Exflagellation	Ookinete	Midgut invasion	Oocyst	Sporozoite formation	Salivary gland infection	Transmitted by bite
Genus	Species							
(*Myzomia*)	*culicifaces*[j]				−			
	fluviatilis[j]				−			
	jeyporensis[j]				−			
	maculatus[x,j]				−			
	splendidus[j]				−			
	stephensi[y,j,e,f]		+	−	−			
	stephensi[g]**	+	+	+	+	+	+	+

Names in brackets are old names used by investigators (e.g. *Aedes aegypti* is the modern name for *Stegomyia fasciata*). *Could be selected to be extremely susceptible. **Parasite selected for transmissibility, Corradetti et al. (1966).

[a] Brumpt (1935).
[b] Brumpt (1936).
[c] Huff (1965).
[d] Mohan (1955).
[e] Russell (1942).
[f] Shahabuddin et al. (1995).
[g] Alavi et al. (2003).
[h] Dasgupta and Ray (1956).
[i] Inoki (1951).
[j] Russell (1942).
[k] Weathersby (1962).
[l] Okpala (1958).
[m] Cantrell and Jordan (1945).
[n] Trembley (1946).
[o] Roubaud et al. (1939).
[p] Cantrell and Jordan (1949).
[q] Cerqueira and Paraense (1945) and Paraense (1945).
[r] Singh and Mohan (1955).
[s] Vargas and Beltran (1941).
[t] Eyles (1952).
[u] Eyles (1960).
[v] Vernick (1995).
[w] Haas and Akins (1947).
[x] Hunninen (1953).
[y] Rudin et al. (1991).

4. The ookinete and midgut invasion

The ookinete has been an object of fascination to parasitologists for over a century. McCallum (1898) first described its gliding motility, thereafter its penetration of the peritrophic matrix and midgut epithelium were recorded in surprising detail by Indacochea (1935) and Freyvogel (1966). Following the ultrastructural descriptions of ookinete formation and development (Garnham et al., 1962; Canning and Sinden, 1973; Mehlhorn et al., 1980) and the concomitant events of meiosis (Sinden et al., 1985), the absence of methods for large-scale ookinete culture hindered molecular analysis. Nevertheless monoclonal antibody techniques identified the potent surface antigens P25 and P28, and their development as transmission-blocking antigens remains a jewel in the crown of antimalarial vaccine development (Kaslow, 1994; Miles et al., 2002; Hisaeda and Yasutomo, 2002). Gene-by-gene analysis of the ookinete has described a score or more ookinete molecules, mostly surface and secreted proteins (Table 3). Antibodies to many of the micronemal proteins identified by this means also have transmission-blocking activity, these antigens include CTRP, chitinase, WARP and SOAP.

5. The application of proteomics

In our efforts to understand parasite biology more completely we have recently undertaken a MudPIT proteomic analysis of the *P. berghei*life stages (Hall et al., 2004). One thousand eight hundred and thirty-six of the 5000–6000 direct gene products encoded in the genome have been identified with high probability, and their expression throughout the life cycle determined. This has allowed us to describe, in part, some of the developmental strategies of the parasite (Florens et al., 2002; Lasonder et al., 2002; Hall et al., 2004). Here I will focus on just two stages of the *P. berghei* life cycle, the gametocyte and the ookinete.

5.1. The gametocyte proteome

To determine which molecules of those detected might be unique to the sexual stages we compared the proteome of the gametocytes with their preceding and subsequent stages of development, namely the asexual blood stages and the ookinete. An overview of the 733 gametocyte

Table 2
Reported development of *Plasmodium berghei* in anopheline and culicine mosquitoes

Mosquito		Exflagellation	Ookinete	Migut Invasion	Oocyst	Sporozoite formation	Salivary gland infection	Transmitted by bite
Genus	Species, strain							
Anopheles	*dureni*[a,b]		+		+		++	+
	stephensi[b,c,d]	+	+	+	+	+	+	+
	gambiae[c,d]	+	+	+	+	+	+	+
	gambiae'A'							
	LSW5 (white eye)[e]		+died		−	−		
	3P6RR[e]		+		+	+		
	16CSS[e]		+		+			
	PALA[e]		+		+	−		
	atroparvus[c]							+
	quadrimaculatus[f,g,h]		+		+		rare	+sporadic
	freeborni[f,h]				+		rare	
	bradleyi[f,h]				+		rare	
	aztecus[b,f,h]		+		+		rare	
	crucians[g]				−			+
	punctipennis[g]							+
	atropus[g]				−			
	albimanus[g]				−			
	sinensis[j]				−		−	−
Aedes	*aegypti*[g,b,h,i,d]	+	+	−	−	−	−	−
	albopictus[j]				−		−	−
	togoi[j]				−		−	−
	japonicus[j]				−		−	−
	taeniorhynchus[g]				−			
Armigeres	*subalbatus*[j]				−		−	−
Culex	*nigripalpus*[g]				−			
	pipienspallens[j]				−		−	−
	tritaeniorhynchus[j]				−		−	−
	bitaeniorhynchus[j]				−		−	−
	hayashi[j]				−		−	−
	quinquefasciatus[f,h]				−			
	salinarius[b]		−		−		−	

[a] Vincke and Peeters (1953).
[b] Yoeli (1973).
[c] Yoeli et al. (1965), Al-Mashhadani et al. (1980) and Cornelissen et al. (1979).
[d] Alavi et al. (2003).
[e] Hilton (1974).
[f] Vanderberg and Yoeli (1965) and Yoeli (1973).
[g] Nayar and Young (1984).
[h] Celaya et al. (1956).
[i] Raffaele and Baldi (1950).
[j] Weathersby (1962) (note that this study lacks a 'positive control').

proteins discovered, revealed that 436 were found in all three samples and were defined as 'housekeeping' proteins. Seventy-two were found in the asexual stages and gametocyte, and may reflect life within the red cell. One hundred and twenty-seven were unique to the gametocytes and therefore function during the processes of gametocytogenesis or gametogenesis. Seventy-six were shared with the ookinete, these may represent proteins involved in sexual development that persist into the ensuing ookinete.

Examining the known properties and functions of proteins in each of the above categories reassuringly revealed amongst the gametocyte-specific groupings six members of the P48/45 family of surface antigens; P377 that has been located in the gametocyte-specific osmiophilic bodies that are secreted during escape from the red cell; numerous signalling pathway components including protein kinases of which one is cGMP-dependent; and a wide range of microtubule/axoneme related components. Whereas α

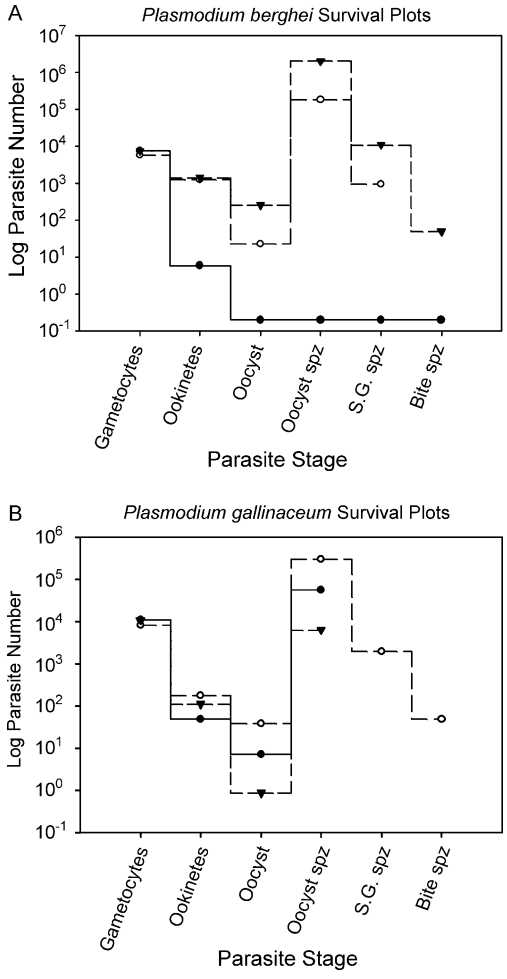

Fig. 1. Survival plots for a *Plasmodium berghei* (A) and *Plasmodium gallinaceum* (B) in *Aedes aegypti*, *Anopheles stephensi* and *Anopheles gambiae*. Parasite attrition is significant in all three mosquito species, reflecting the efficacy of the varying genetic and immune barriers raised in each parasite–vector combination (based on data from Alavi et al. (2003)).

and β tubulin (the key constituents of microtubule protofilaments) are highly expressed in all life stages, a tubulin-specific chaperone is most highly expressed in the gametocyte. It is difficult not to suggest that this chaperone functions in the maintenance or transformation of the very large pool of monomeric tubulin in the microgametocyte that, within 15 s of entering the mosquito gut, polymerises into complex patterned organelles, including mitotic spindles and fully formed kinetosomes/axonemes (Sinden et al., 1976). Consistent with the activated microgametocyte and microgametes being the only stages to form motile axonemes it is no surprise that we find numerous dynein-related proteins, uniquely expressed in these cells.

5.2. The ookinete proteome

When examining the ookinete, we have found it informative to compare and contrast all three invasive-stages, the merozoite (represented by a mixture of all asexual blood stages); the sporozoite and the ookinete. Unfortunately the comparison with the sporozoite is heavily constrained by the low number of proteins we have identified in these cells in the rodent malaria parasites. Of the 1092 proteins found in the ookinete 307 are unique to this stage, 10 are shared by all three groups; 210 are shared only with the asexual stages (this will fall as more sporozoite proteins are identified as housekeeping/strategy-related groups); and nine are shared only with the sporozoite—the residual 546 ookinete proteins are shared with other life stages (notably gametocytes).

Table 3
Summary of the known proteins and their location in the malaria ookinete prior to proteomic studies (summarised from Sinden et al. (2004))

Organelle system	Location	Protein Name
Pellicle	*Plasmalemma*	P25, P28a/b, P89, P87,P75, P52, P43
	Inner membrane complex	Myosin, actin, IMC-1
Apical complex	*Apical ring?*	P155
	Collar	CHT
	Micronemes	CTRP, WARP, SOAP, CHT1, CHT2, Sub2
Endoplasmic reticulum		P39
Cytoplasm		Caspase 3
Unknown location		D11, PLAP-1, Pg17, Pg32, Pb105, Pb16

When comparing the invasive stage proteomes what is clearly demonstrated are the broad groupings of co-expressed proteins that can be identified, and how these often fall into logical functional, or organelle-related groupings. Thus cell motor proteins, required to move organelles in all life stages are shared, whereas one motor protein, actin 3 is unique to the ookinete. The ookinete is, coincidentally, unique in that the apical prominence and collar are reportedly protruded/retracted during the invasive process (Garnham et al., 1962). An interesting observation in the current dataset, is the detection of a subset of inner membrane complex proteins (e.g. IMC-1b,c) only in the ookinete, yet all three invasive stages possess an inner membrane complex (IMC). When viewed by helium freeze-fracture techniques the ookinete at present is unique in possessing large pores in the IMC (Raibaud et al., 2001). The structure and composition of the cytoskeleton of the motile stages clearly require more detailed and comparative study. One of the most revealing of the current observations relates to the organisation of the secretory organelles. The merozoite (asexual blood stages) expresses rhoptry proteins in abundance (e.g. members of the RAP and RhopH complex proteins). Many of these proteins are expressed in the sporozoite but only one was detected in the ookinete (probably due to bloodstage contamination). Conversely, in the ookinete it is the micronemal proteins that predominate (e.g. CTRP, SOAP, WARP, chitinase, and Perforin-Like

Proteins (PLP-3 and 4)). Micronemal proteins are also found in the sporozoite (e.g. TRAP—the CTRP analogue, and PbPLP-1). This observation gives weight to the idea that the ookinete, uniquely amongst the malarial invasive stages, does not have rhoptries, which in turn correlates with the now widely recognised fact that the ookinete does not form a parasitophorous vacuole in the invaded host cell. The malarial sporozoite represents a 'pluripotential zoite' in that both micronemes and rhoptries are well developed. In the malarial sporozoite the micronemes may mediate invasion and traversal of the salivary gland cell, of the Kupffer cell and some hepatocytes (Mota and Rodriguez, 2001; Ishino et al., 2004), whereas the rhoptry mediates formation of a parasitophorous vacuole in the 'hepatocyte-of-residence'. If this is the case it will be interesting to unravel the signalling cascade regulating exocytosis of the microneme and rhoptry contents.

Expression of invasive stage surface proteins has, with the single exception of MSP-1, reinforced the established concept of the stage specificity of expression, a logical concept that suggests the surface structure is uniquely adapted to the host environment, and the specific targeting of the parasite to different host cells. Typical examples of such proteins are: MSP-7–9 in the merozoite; P25 and P28 in the ookinete and CSP in the sporozoite. MSP-1 expression, though heavily upregulated in the bloodstages, is detected at low abundance even in oocyst and sporozoite preparations, where bloodstage contamination is not possible. We are reassured by the observation of Le Roch et al. (2003) who detected transcription of the MSP-1 gene in the sporozoite of *P. falciparum.* Whether this reflects a conserved role for MSP-1 on the plasmalemma of all life stages or 'leaky' expression, remains to be determined.

Using the above proteomic data in conjunction with the known structure of the pellicle of the ookinete (Sinden, 1978; Raibaud et al., 2001) and of the molecular motor so elegantly described in the malaria sporozoite (Kappe et al., 2004) and in *Toxoplasma* tachyzoites (Opitz and Soldati, 2002), we can now propose a preliminary model for the molecular organisation of the essential supramolecular machines that make up this structure (Fig. 2). Putative interactions between molecules are illustrated in the central section of the figure. Orphan molecules with no known interrelationships are 'stored' on the left of the picture. Clearly it is now important to assemble the 'orphan'

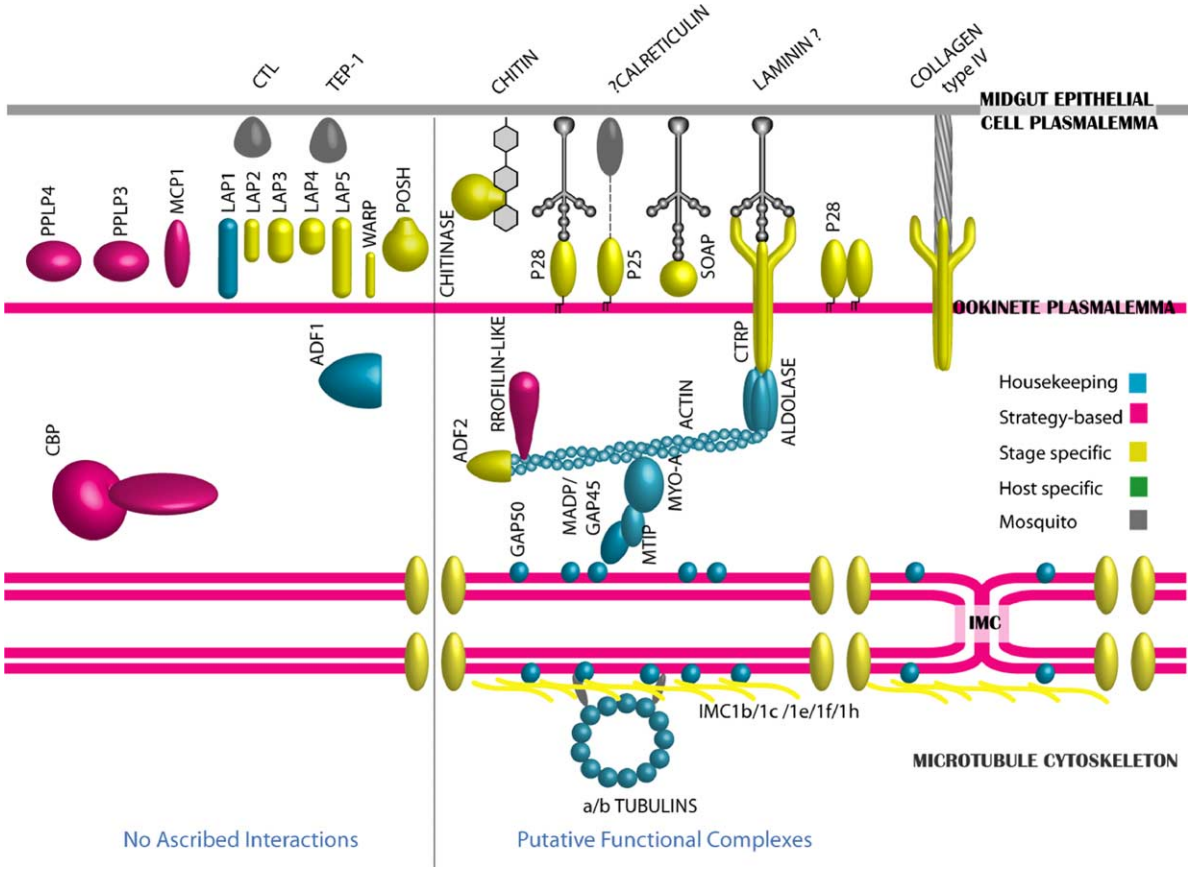

Fig. 2. Diagram illustrating a possible organisation of the ookinete pellicle including the molecular motor. Molecules on the left of the illustration have as yet no proven structural associations with the possible 'supramolecular complex' envisaged. The orientation of the midgut epithelial structures is topologically incorrect and for illustrative purposes only.

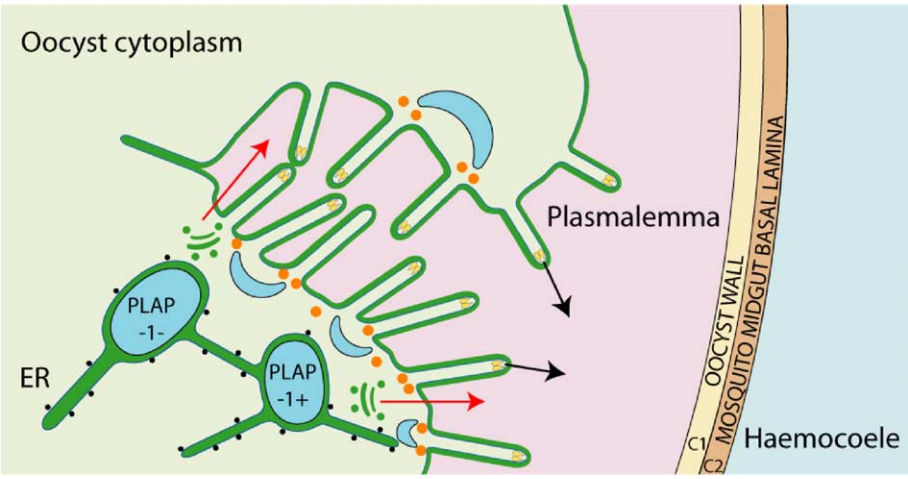

Fig. 3. Theoretical model for PLAP1 expression in heterokaryotic oocyst derived from a cross fertilisation of a PLAP1 knockout line and wild-type parasite. LAP1(green) is synthesised in the ER, passes through the nuclear envelope and Golgi to be transported in vesicles to the oocyst surface at the time of sporoblast and sporozoite formation. Red arrows indicate simple vesicular traffic to the plasmamembrane. Black arrows indicate possible transport through the micronemes/rhoptry vesicle system.

molecules into the emergent supramolecular complexes to determine exactly how the ookinete functions and interacts with its insect host.

Our understanding of the proteome of the ookinete surface has increased significantly. Amongst these molecules is PLAP-1 a molecule with multiple adhesive and putatively immunoregulatory domains. In *P. berghei* the protein, a secreted molecule with no membrane anchor, is detected in all life stages, whereas in *P. falciparum* it has been detected only in the gametocytes (of the bloodstages examined) where IFAT studies demonstrate PfLAP-1 is primarily found in a perinuclear location and in the parasitophorous vacuole. In *P. berghei* the protein is clearly located on the surface of the free sporozoite but was difficult to demonstrate on the surface of other stages, paradoxically including the ookinete where protein expression levels are highest. Claudianos et al. (2002) showed that, despite constitutive transcription of the gene, a knockout of PbLAP-1 was lethal only in the oocyst stage, in which sporozoite formation and cytokinesis were suppressed—very much reminiscent of the circumsporozoite protein (CSP)-knockout phenotype (Menard et al., 1997). Trueman et al. (2004) subsequently demonstrated that the PbLAP-1 knockout could be complemented in the polyploid oocyst stage by cross-fertilization with a wild-type parasite. This 'comple-complementation' could not be reproduced by co-infection of mosquitoes with mixed ookinetes of the two parental lines. Thus PLAP-1, a secreted molecule, cannot function between separated oocysts, i.e. it does not function in the haemocoele environment. One possible hypothesis based on the above data is to conclude that the molecule is secreted into the endoplasmic reticulum, passes through the nuclear envelope to the Golgi apparatus (Hager et al., 1999) and is secreted through regulated vesicles to the parasite cell surface. In the *P. falciparum* gametocyte such vesicles could include the osmiophilic bodies, in the dividing oocyst the vesicles could be either the numerous small vesicles seen to fuse with the oocyst plasmalemma (Sinden, 1978) or the micronemes/rhoptries of the sporozoites—the route by which CSP reaches the parasite surface (Fig. 3). Whilst the domain structure of LAP-1 suggests it interacts readily with other surface proteins, and a putative role in immunoregulation has been proposed we must recognise that the true function(s) of this protein remains unknown.

Clearly the explosive development of a wide range of experimental methods to analyse parasite structure, to manipulate and observe gene expression in the living cell have revolutionised our understanding of parasite function, many researchers are now integrating these approaches into the emergent concept of 'Systems Biology'. But powerful as these approaches may be they are only relevant if they are considered in the full context of parasite biology within its hosts. It is the older literature that contains a vast wealth of relevant biological data that forms the background of the picture into which we wish to place our subject of interest. Recognition of this fact will accelerate the formulation of relevant questions and the correct conclusions being reached. It is this integration of the old and the new data that to my mind represents the real systems-biology. Failure to recognise the old literature may introduce spurious novelty, speed publication and please editors, but it will only slow down progress.

Acknowledgements

The author would like to thank Prof. Yates, Drs Florens, Carucci, Hall and Carlton for their generous collaboration.

Mr J.D. Raine for the analysis of the *P. berghei* proteome, and past members of the lab. Our work has received sustained financial support from The Wellcome Trust, The European Union, The Malaria Vaccine Initiative, The BBSRC, and the World Bank/UNDP/World Health Organisation.

References

Alavi, Y.I.H., Arai, M., Mendoza, J., Tufet-Bayona, M., Sinha, R., Fowler, K., Billker, O., Franke-Fayard, B., Janse, C.J., Waters, A., Sinden, R.E., 2003. The dynamics of interactions between *Plasmodium* and the mosquito: a study of the infectivity of *P. berghei* and *P. gallinaceum* to *Anopheles stephensi*, *A. gambiae* and *Aedes aegypti*. Int. J. Parasitol. 33, 933–943.

Al-Mashhadani, H.M., Davidson, G., Curtis, C.F., 1980. A genetic study of the susceptibility of *Anopheles gambiae* to *Plasmodium berghei*. Trans. R. Soc. Trop. Med. Hyg. 74, 585–594.

Anderson, R.M., May, R.M., Gupta, S., 1989. Non-linear phenomena in host–parasite interactions. Parasitology 99, S59–S79.

Arai, M., Billker, O., Sinden, R.E., 2001. Both mosquito- and host blood-derived xanthurenic acid induce gametogenesis of *Plasmodium gallinaceum* in the midgut of *Aedes aegypti*. Mol. Biochem. Parasitol. 116, 17–24.

Billker, O., Lindo, V., Panico, M., Etienne, A.E., Paxton, T., Dell, A., Rogers, M., Sinden, R.E., Morris, H.R., 1998. Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. Nature 392, 289–292.

Billker, O., Miller, A.J., Sinden, R.E., 2000. Determination of mosquito bloodmeal pH in situ by ion-selective microelectrode measurement: implications for the regulation of malarial gametogenesis. Parasitology 120, 547–551.

Billker, O., Deschamps, S., Tewari, R., Wenig, G., Franke-Fayard, B., Brinkmann, V., 2004. Calcium and calcium dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. Cell 117 (4), 503–514.

Blandin, S., Shiao, S.H., Moita, L.F., Janse, C.J., Waters, A.P., Kafatos, F.C., Levashina, E.A., 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. Cell 116, 661–670.

Bruce-Chwatt, L.J., 1985. Essential malariology, 2nd ed. Heinemann Medical Books, London.

Brumpt, E., 1935. Récéptivite de divers oiseaux domestiques et savages au parasite (*Plasmodium gallinaceum*) du paludisme de la poule domestique. Transmission de cet hematozoaire par le moustique *Stegomyia fasciata*. Comptes Rendus hebdomadaires des séances de l'Académie des sciences 200, 783–967.

Brumpt, E., 1936. Étude experimentale du *Plasmodium gallinaceum* parasite de la poule domestique. Transmission de ce germe par *Stegomyia fasciata* et *Stegomyia albopicta*. Annales de Parasitologie Humaine et Comparée 14, 597–620.

Canning, E.U., Sinden, R.E., 1973. The organization of the ookinete and observations on nuclear division in oocysts of *Plasmodium berghei*. Parasitology 67, 29–40.

Cantrell, W., Jordan, H.B., 1945. New mosquito hosts for *Plasmodium gallinaceum*. J. Parasitol. 31, 55–56.

Cantrell, W., Jordan, H.B., 1949. Relative susceptibility of *Aedes aegypti, Aedes albopictus*, *Aedes canadensis* and *Anopheles quadrimaculatus* to *Plasmodium gallinaceum*. J. Infect. Dis. 85, 170–172.

Celaya, B.L., Box, E.D., Gingrich, W.D., 1956. Infectivity of *Plasmodium berghei* for Anopheles quadrimaculatus and other mosquitoes. Am. J. Trop. Med. Hyg. 5, 168–182.

Cerqueira, N.L., Paraense, W.L., 1945. Uma nova espécie de Aedes, transmissora de *Plasmodium gallinaceum* (Diptera, Culicidae). Memórias do Instituto Oswaldo Cruz 42, 11–15.

Claudianos, C., Trueman, H.E., Dessens, J.T., Arai, M., Mendoza, J., Butcher, G.A., Crompton, T., Sinden, R.E., 2002. A malaria scavenger receptor-like protein essential for parasite development. Mol. Microbiol. 45, 1473–1484.

Collins, F.H., Sakai, R.K., Vernick, K.D., Paskewitz, S., Seeley, D.C., Miller, L.H., Collins, W.E., Campbell, C.C., Gwadz, R.W., 1986. Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. Science 234, 607–610.

Cornelissen, A.W.C.A., van Seventer, H.A., Boorsma, E.G., Kroon, C.C.M., 1979. Studies on susceptibility to *Plasmodium berghei* in a normal vector population of *Anopheles atroparvus* after the introduction of *Plasmodium*-refractory genotypes: the establishment of the vector population with overlapping generations. Acta Liedense 47, 79–88.

Corradetti, A., Dojmi, G.L., Palmieri, C., 1966. Trasmissione Di *Plasmodium Gallinaceum* Con *Anopheles Stephensi*. Atti Della Societa Peloritana Di Scienze Fisiche Matematiche e Naturali XII, 1–11.

Dasgupta, B., Ray, H.N., 1956. Aedes albopictus as a vector of *Plasmodium gallinaceum*. Bull. Calcutta School of Trop. Med. 4, 63.

Eyles, D.E., 1952. Studies on *Plasmodium gallinaceum*.IV. A comparison of the susceptibility of *Aedes aegypti*, *Anopheles quadrimaculatus* and *Anopheles freeborni*. Am. J. Hyg. 56, 71–77.

Eyles, D.E., 1960. Susceptibility of *Anopheles albimanus* to primate and avian malarias. Mosquito News 20, 368–371.

Florens, L., Washburn, M.P., Raine, J.D., Anthony, R.M., Grainger, M., Haynes, J.D., Moch, J.K., Muster, N., Sacci, J.B., Tabb, D.L., Witney, A.A., Wolters, D., Wu, Y., Gardner, M.J., Holder, A.A., Sinden, R.E., Yates Iii, J.R., Carucci, D.J., 2002. A proteomic view of the Plasmodium falciparum life cycle. Nature 419, 520–526.

Freyvogel, T.A., 1966. Shape, movement in situ and locomotion of plasmodial ookinetes. Acta Tropica 23, 201–221.

Garnham, P.C.C., Bird, R.G., Baker, J.R., 1962. Electron microscopic studies of motile stages of malarial parasites III. The ookinetes of *Haemamoeba* and *Plasmodium*. Trans. R. Soc. Trop. Med. Hyg. 56, 116–120.

Haas, V.H., Akins, H., 1947. Transmission of *Plasmodium gallinaceum* by *Anopheles quadrimaculatus*. J. Nat. Malaria Soc. 6, 244–245.

Hager, K.M., Striepen, B., Lewis, L.G., Roos, D.S., 1999. The nuclear envelope serves as an intermediary between the ER and Golgi complex in the intracellular parasite *Toxoplasma gondii*. J. Cell Sci. 112, 2631–2638.

Hall N., Karras M., Raine J.D., Carlton J.M., Kooij T.W.A., Berriman M., Florens L., Janssen C.S., Pain A., Christophides J.K., James K., Rutherford K., Harris B., Harris D., Churcher C., Quail M.A., Ormond D., Doggett J., Trueman H.E., Mendoza J., Bidwell S.L., Rajandream M-A., Carucci D.J., Yates J.R., Kafatos F.C., Janse C.J., Barrell B., Turner C.M.R., Waters A.P., Sinden R.E., 2004. A global analysis of the molecular life-strategies of malaria parasites by integrated genomic, transcriptomic and proteomic analyses of both vertebrate and mosquito stages. Science (in press)

Hay, S.I., Rogers, D.J., Randolph, S.E., Stern, D.I., Cox, J., Shanks, G.D., Snow, R.W., 2002. Hot topic or hot air? Climate change and malaria resurgence in East African highlands. Trends Parasitol. 18, 530–534.

Hilton, D.F.J., 1974. Resistance and susceptibility of some strains of the *Anopheles gambiae* complex to infections with *Plasmodium* spp. of rodents. Mosquito News 34, 81–85.

Hisaeda, H., Yasutomo, K., 2002. Development of malaria vaccines that block transmission of parasites by mosquito vectors. J. Med. Invest. 49, 118–123.

Huff, C.G., 1965. Susceptibility of mosquitoes to avian malaria. Exp. Parasitol. 16, 107–132.

Hunninen, A.V., 1953. Comparative development of Plasmodium relictum oocysts in *Anopheles quadrimaculatus*, *A. albimanus* and *Culex pipiens*. J. Parasitol. 39, 28–32.

Indacochea, A.A., 1935. La penetracion del ookineto del Plasmodium falciparum en el epitelio intestinal del Anopheles maculipennis. Rivista Di Malariologia XIV, 3–6.

Inoki, S., 1951. Studies on the exoerythrocytic schizogony of the malarial parasite exoerythrocytic forms of the bird malarial parasites. Med. J. Osaka Univ. 2, 61–73.

Ishino, T., Yano, K., Chinzei, Y., Yud, M., 2004. Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. PLoS Biol. 2, E4. Epub.

Kappe, S.H.I., Buscaglia, C.A., Bergman, L.W., Coppens, I., Nussenzweig, V., 2004. Apicomplexan gliding motility and host cell invasion: overhauling the motor model. Trends Parasitol. 20, 13–16.

Kaslow, D., 1994. Progress toward a transmission-blocking vaccine, Molecular Immunological Considerations in Malaria Vaccine Development. CRC Press, Boca Raton pp. 209–244.

Kawamoto, F., Alejo Blanco, R., Fleck, S.L., Kawamoto, Y., Sinden, R.E., 1990. Possible roles of Ca-2+ and cGMP as mediators of the exflagellation of *Plasmodium berghei* and *Plasmodium falciparum*. Mol. Biochem.Parasitol. 42, 101–108.

Lasonder, E., Ishihama, Y., Andersen, J.S., Vermunt, A.M.W., Paln, A., Sauervein, R.W., Eling, W.M.C., Hall, N., Waters, A.P., Stunnenberg, H.G., Mann, M., 2002. Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. Nature. 419, 537–542.

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

McCallum, W.G., 1898. On the haematozoan infections of birds. J. Exp. Med. 3, 117–136.

Mehlhorn, H., Peters, W., Haberkorn, A., 1980. The formation of kinetes and oocyst in *Plasmodium gallinaceum* (Haemosporidia) and considerations on phylogenetic relationships between Haemosporidia, Piroplasmida and other Coccidia. Protistologica 16, 135–154.

Menard, R., Sultan, A.A., Cortes, C., Altszuler, R., van Dijk, M.R., Janse, C.J., Waters, A.P., Nussenzweig, R.S., Nussenzweig, V., 1997. Circumsporozoite protein is required for development of malaria sporozoites in mosquitoes. Nature 385, 336–340.

Miles, A.P., Zhang, Y., Saul, A., Stowers, A.W., 2002. Large-scale purification and characterization of malaria vaccine candidate antigen Pvs25H for use in clinical trials. Protein Express. Purif. 25, 87–96.

Mohan, B.N., 1955. Comparative susceptibility of some *Aedes* mosquitoes to *Plasmodium gallinaceum*. Indian J. Malariol. 9, 75–79.

Mota, M.M., Rodriguez, A., 2001. Migration through host cells by apicomplexan parasites. Microbes Infect. 3, 1123–1128.

Nayar, J.K., Young, M.D., 1984. Susceptibility of *Anopheles punctipennis* and other Florida mosquitoes to *Plasmodium berghei*. J. Parasitol. 70, 192–194.

Ogwang, R., Mwangi, J., Gachihi, G., Nwachukwu, A., Roberts, C.R., Martin, S.K., 1993. Use of pharmacological agents to implicate a role for phosphoinositide hydrolysis products in malaria gamete formation. Biochem. Pharmacol. 46, 1601–1606.

Okpala, I., 1958. Transmission of *Plasmodium gallinaceum* through *Aedes stokesi*. West African Med. J. 7, 195–196.

Opitz, C., Soldati, D., 2002. The glideosome: a dynamic complex powering gliding motion and host cell invasion by *Toxoplasma gondii*. Mol. Microbiol. 45, 597–604.

Osta, M., Christophides, G.K., Kafatos, F.C., 2004. Effects of mosquito genes on *Plasmodium* development. Science. 303, 2030–2032.

Paraense, W.L., 1945. A transmissão de *Plasmodium gallinaceum* pelo Aedes (Ochlerotatus) lepidus. Memórias do Instituto Oswaldo Cruz 42, 81–84.

Paton, M.G., Barker, G.C., Matsuoka, H., Ramesar, J., Janse, C.J., Waters, A.P., Sinden, R.E., 1993. Structure and expression of a conserved and post-transcriptionally regulated gene encoding a surface protein of the sexual stages from malaria parasite *Plasmodium berghei*. Mol. Biochem. Parasitol. 59, 263–275.

Quakyi, I.A., Matsumoto, Y., Carter, R., Udongsampetch, R., Sjolander, A., Berzins, K., Perlmann, P., Aikawa, M., Miller, L.H., 1989. Movement of a falciparum malaria protein through the erythrocyte cytoplasm to the erythrocyte membrane is associated with lysis of the erythrocyte and release of gametes. Infect. Immun. 57, 833–839.

Raffaele, G., Baldi, A., 1950. Sulla morfologia e sulla transmissione di *P. berghei* (Vinke e Lips). Riv. di malarial. 29, 341–347.

Raibaud, A., Lupetti, P., Paul, R.E., Mercati, D., Brey, P.T., Sinden, R.E., Heuser, J.E., Dallai, R., 2001. Cryofracture electron microscopy of the ookinete pellicle of *Plasmodium gallinaceum* reveals the existence of novel pores in the alveolar membranes. J. Struct. Biol. 135, 47–57.

Roubaud, E., Colas-Belcour, J., Mathis, M., 1939. Transmission de *Plasmodium gallinaceum* par Aedes geniculatus. Bull. Soc. Path. Exotique 32, 28–30.

Rudin, W., Billingsley, P.F., Saladin, S., 1991. The fate of *Plasmodium gallinaceum* in *Anopheles stephensi* Liston and possible barriers to transmission. Ann. Soc. Belge Med. Trop. 71, 167–177.

Sattabongkot, J., Maneechai, N., Rosenberg, R., 1991. *Plasmodium vivax*: gametocyte infectivity of naturally infected Thai adults. Parasitology 102, 27–31.

Shahabuddin, M., Kaidoh, T., Aikawa, M., Kaslow, D.C., 1995. *Plasmodium gallinaceum*: mosquito peritrophic matrix and the parasite–vector compatibility. Exp. Parasitol. 81, 386–393.

Sinden, R.E., 1978. Cell Biology, in: Killick-Kendrick, R., Peters, W.A. (Eds.), Rodent Malaria. Academic Press, New York, pp. 85–186.

Sinden, R.E., Canning, E.U., Spain, B., 1976. Gametogenesis and fertilization in *Plasmodium yoelii nigeriensis*: a transmission electron microscope study. Proc. R. Soc. (Lond) B. 193, 55–76.

Sinden, R.E., Hartley, R., Winger, L., 1985. The development of *Plasmodium* ookinetes in vitro: an ultra-structural study including a description of the meiotic division. Parasitology 91, 227–244.

Sinden, R.E., Alavi, Y.I.H., Butcher, G.A., Dessens, J.T., Raine, J.D., Trueman, H.E., 2004. Ookinete Cell Biology, in: Waters, A.P., Janse, C.J. (Eds.), Genomes and the Molecular Cell Biology of Malaria Parasites. Caister Academic Press, Wymondham, UK, pp. 475–500.

Singh, J., Mohan, B.N., 1955. Susceptibility of *Culex* (*Culex*) bitaeniorhynchus Giles, 1901, to *Plasmodium relictum* but not to *Plasmodium gallinaceum* and *Plasmodium falciparum*. Indian J. Malariol. 9, 71–74.

Trembley, H.L., 1946. *Aedes atropalpus* (Coq.), a new mosquito vector of *Plasmodium gallinaceum* Brumpt. J. Parasitol. 32, 499–501.

Trueman, H.E., Raine, J.D., Florens, L., Dessens, J.T., Mendoza, J., Johnson, J., Waller, C.C., Delrieu, I., Holder, A.A., Langhorne, J., Carucci, D.J., Yates, J.R., II, ., Sinden, R.E., 2004. Functional characterisation of an LCCL/lectin-domain containing protein family in *Plasmodium berghei*. J. Parasitol. 2004; in press.

Vanderberg, R., Yoeli, M.J., 1965. Some physiological and metabolic problems related to maintenance of the *Plasmodium berghei* cycle in *Anopheles quadrimaculatus*. Ann Soc. Belge. Med. Trop. 45, 419–426.

Vargas, L., Beltran, E., 1941. *Culex quinquefasciatus*, a new vector of *Plasmodium gallinaceum*. Science 94, 389–390.

Vernick, K.D., Fujioka, H., Seeley, D.C., Tandler, B., Aikawa, M., Miller, L.H., 1995. *Plasmodium gallinaceum*: a refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. Exp. Parasitol. 80, 583–595.

Vincke, I.H., Peeters, E., 1953. Observations sur la transmission de sporozoites d'*Anopheles dureni* à des rongeurs sauvages et de laboratoire. Ann. Soc. Belge. Med Trop. 33, 87–93.

Weathersby, A.B., 1962. Susceptibility of certain Japanese mosquitoes to *Plasmodium gallinaceum* and *Plasmdoium berghei*. J. Parasitol. 48, 607–609.

Yoeli, M., 1973. *Plasmodium berghei*: mechanisms and sites of resistance to sporogonic development in different mosquitoes. Exp. Parasitol. 34, 448–458.

Yoeli, M., Vanderberg, J., Nawrot, R., Most, H., 1965. Studies on sporozoite-induced infections of rodent malaria. II. *Anopheles stephensi* as an experimental vector of *Plasmodium berghei*. Am. J. Trop. Med. Hyg. 14, 927–930.