Plasmodium

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Plasmodium* is a genus of parasitic protozoa which infect erythrocytes of vertebrates and cause malaria. Their life cycle alternates between mosquito and vertebrate hosts. Parasites enter the bloodstream after a mosquito bite, and multiply sequentially within liver cells and erythrocytes before becoming male or female sexual forms. When ingested by a mosquito, these fuse, then the parasite multiplies again to form more invasive stages which are transmitted back in the insect's saliva to a vertebrate. All invasive stages have specialized secretory structures (apical organelles) typical of the protozoan subphylum Apicomplexa, enabling them to invade cells and tissues. Parasites exploit erythrocytes by ingesting haemoglobin and exporting molecules which change erythrocyte membrane properties. Five species infect humans, the most lethal being *Plasmodium falciparum* which can cause pathology and death by clogging blood vessels in brain, viscera and placenta. Many hundreds more species infect other mammals, birds and lizards.

Introduction and Description of *Plasmodium*

Parasites of the genus *Plasmodium* are protozoans which invade and multiply within erythrocytes of vertebrates, and are transmitted by mosquitoes. The motile invasive stages (merozoite, ookinete and sporozoite) are elongate, uninucleate cells able to enter cells or pass through tissues, using specialized secretory and locomotory organelles. Intracellular stages live in a membrane-lined cavity (parasitophorous vacuole) within the host cell cytoplasm. The genus is currently classified on the basis of molecular and other evidence as: Kingdom Protozoa, Subkingdom Biciliata, Infrakingdom Alveolata, Phylum Myzozoa, Subphylum Apicomplexa, Class Aconoidasida, Order Haemosporina, Genus *Plasmodium* (Cavalier-Smith, 2003). The Subphylum Apicomplexa comprises nearly 5000 described species, all parasitic including several genera of medical and economic importance including, besides *Plasmodium*, *Babesia*, *Toxoplasma*, *Cryptosporidium*, *Theileria*, *Eimeria* and *Isospora*. They all lack cilia and flagella except for the microgametes, but possess invasive organelles (rhoptries, micronemes and polar rings) constituting the apical complex, structures diagnostic for this group (hence the name Apicomplexa). They also typically contain one or more mitochondria, and an elongate membranous organelle known as the apicoplast, and move by a unique form of gliding locomotion. Evolutionarily, the nearest relatives of this group are the ciliates and dinoflagellates. **See also**: Alveolates; Apicomplexa; Babesiosis; Cryptosporidiosis; *Eimeria*; *Plasmodium*; Protozoa; Protozoan Evolution and Phylogeny; Protozoan

Plasmodium Hosts

Malaria, an infectious disease associated with fever, anaemia and other pathologies, is caused by species of *Plasmodium*. This genus infects mammals, birds and lizards, and is transmitted by the bite of female mosquitoes (*Anopheles* species in mammals, or *Culex* species in birds and lizards) in which part of its life cycle is spent. Mammalian malarias are confined to antelopes, lemurs, bats, rodents and primates (including humans), and absent in felids, canids, equids or bovids, for unknown reasons. In humans there are four major species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*), but recently another, *Plasmodium knowlesi*, whose usual host is the Kra monkey, has been found endemic in humans in parts of South-East Asia (Cox-Singh and Singh, 2008). There is also molecular evidence of other as yet poorly defined species responsible for human malaria. Most deaths are due to the malignant tertian malaria parasite, *P. falciparum*, although the benign tertian *P. vivax* is an important pathogen especially in Asia and Latin America. It is estimated that in 2006 there were approximately 3.3 billion people at risk of developing malaria each year, with at least 500 million cases, and nearly a million deaths annually (WHO World Malaria Report, 2008). This averages to one person dying of malaria every 30 s. At present over 90% of deaths occur within the continent of Africa, mainly among young children. **See also**: Malaria; Parasitism: the Variety of Parasites; Protozoan Pathogens of Domestic and Companion Animals; Protozoan Pathogens of Humans

Life Cycle

Plasmodium alternates between vertebrate and mosquito hosts (**Figure 1**), with its sexual phase in the mosquito. The transmissive form, the sporozoite, is injected into the skin of a vertebrate by the female mosquito with anticoagulant saliva, as a preliminary to a blood meal. Sporozoites enter the bloodstream or lymphatics and circulate to infect the liver (mammals) or the spleen, endothelial cells and macrophages (birds and lizards). There they become intracellular and proliferate to form hundreds of invasive merozoites (the pre-erythrocytic or exo-erythrocytic phase). These are released into the bloodstream and invade erythrocytes. Inside an erythrocyte the parasite feeds on its host cell, then multiplies to form more merozoites which exit and invade new erythrocytes, a cycle repeated many times (the asexual blood cycle, **Figure 2**) to amplify numbers greatly. The time from invasion to exit varies with species, 48 h for *P. falciparum* and *P. vivax* and 72 h for *P. malariae* and *P. ovale*, the synchronous release of merozoites coinciding with fever peaks. Eventually a sexual phase begins, the parasite growing inside its host cell into either a female gametocyte (macrogametocyte) or a male gametocyte (microgametocyte). **See also**: Malaria

The life cycle's continuation now depends on gametocytes being taken into the gut of a feeding female mosquito where both types of gametocyte escape from their host cells. Male gametocytes divide rapidly into a number of motile flagellated microgametes each of which can fertilize a female macrogamete to form a zygote (**Figure 1**). The parasite then becomes a motile ookinete, penetrating the mosquito gut wall and encysting as a rounded oocyst. The parasite multiplies asexually within this to form many hundreds of motile sporozoites (sporogony). Mature sporozoites escape through the oocytst wall into the insect's blood cavity (haemocoel) and thence to the salivary glands, penetrating their walls to reach the mosquito's stored saliva in readiness for transmission to a vertebrate at another blood meal. **See also**: Parasitism: Life Cycles and Host Defences against Parasites; Protozoan Sexuality

Asexual Blood Stages

Asexual blood stages include in sequence, the merozoite, ring, trophozoite and schizont stages (see Bannister *et al.*, 2001).

Merozoites

These invasive forms (**Figure 2c** and **Figure 3a–d**) are the smallest stages. They have specialized organelles enabling merozoites to invade erythrocytes without lysing them. Merozoite are oval with a prominence at the anterior end supported by three cytoskeletal rings (polar rings), anchored to which are two sets of secretory organelles, rhoptries and micronemes (Bannister *et al.*, 2000). Rhoptries are pear-shaped vesicles, two in number (**Figure 2c** and **Figure 3a**), whereas micronemes are much smaller and more numerous (20 or more) though similar in shape. The narrow ends of these organelles converge at the merozoite apex, poised for secretion. The name rhoptry refers to their shape (Greek *rhoptos* = a club), and microneme is Greek for 'small thread', which now seems rather misleading. Merozoites also contain small rounded vesicles (dense granules) secreted after invasion, and more elongate ones (exonemes) used in merozoite exit from schizonts. **See also**: Proteases

The merozoite surface is covered by a thick bristly coat, and underlying this are three membranes (collectively, the pellicle), the outer being the plasma membrane and the inner two together forming the inner membrane complex. The actin–myosin motor which propels the merozoite during invasion is located here. Some longitudinal microtubules are attached to the inner layer, anchored anteriorly at the polar rings. Also present are a single nucleus, a mitochondrion, an apicoplast and some ribosomes, the minimal equipment needed for the next, intracellular stage of the cycle.
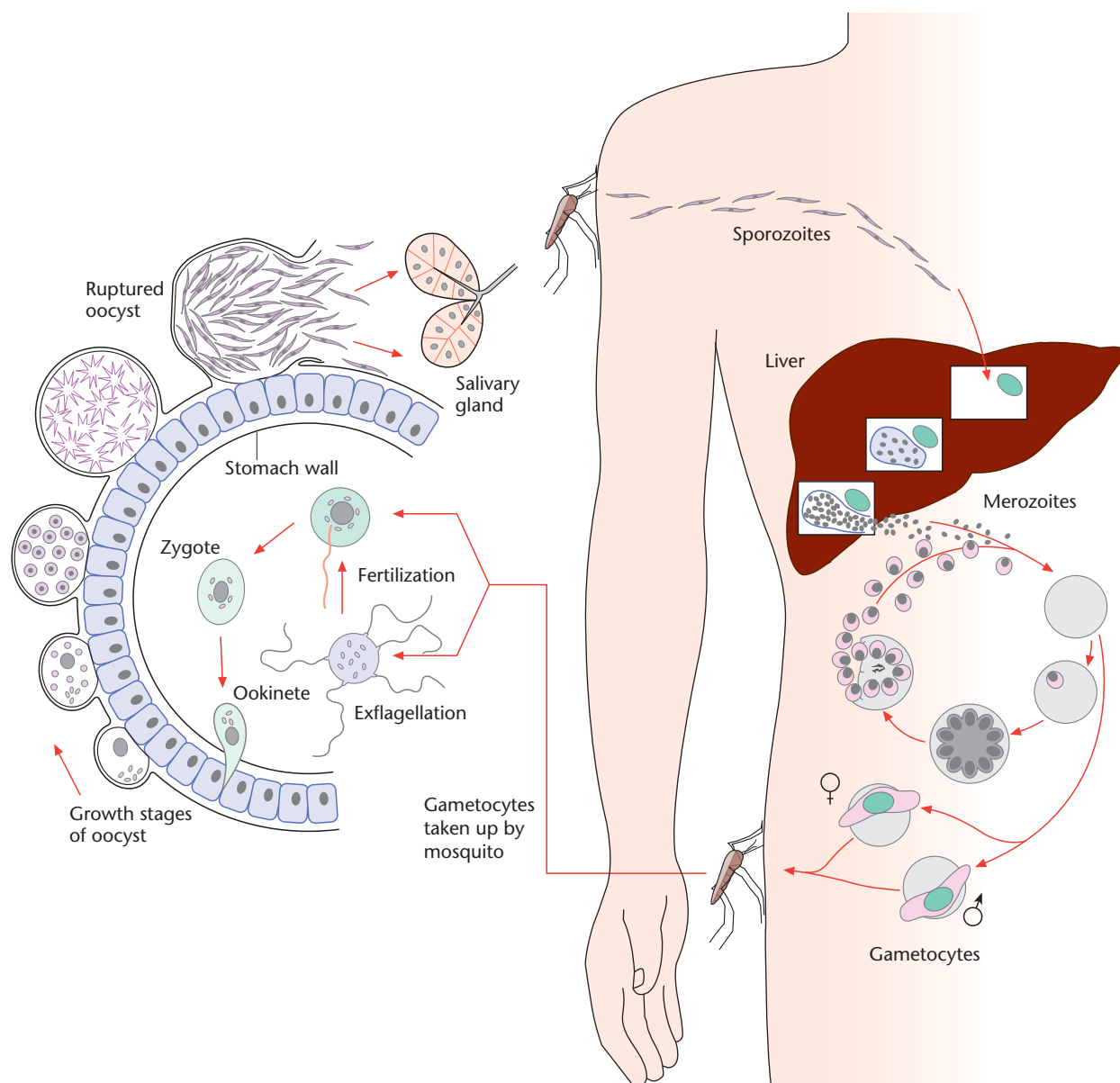
Figure 1   The main features of the life cycle of the malaria parasite *Plasmodium falciparum*, showing its different phases in vertebrate and mosquito hosts.

Invasion of erythrocytes by merozoites

This is a multistage process (**Figure 3b**–**d**), beginning with selective adhesion to the erythrocyte surface then reorientation to bring the merozoite apex into contact with the erythrocyte membrane, forming an irreversible close junction. Secretion from the rhoptries now causes a deep membrane-lined pit to appear in the erythrocyte surface, and into this the parasite moves. The erythrocyte membrane closes over the merozoite to leave it in a membrane-lined space (parasitophorous vacuole) (Ladda *et al*., 1969; Aikawa *et al*., 1978). To bring about these changes, the merozoite secretes from its micronemes and rhoptries a complex cocktail of chemicals, including adhesive proteins

(adhesins), proteases and membrane-altering agents, whose interactions are needed for erythrocyte capture, junction formation, creation of the parasitophorous vacuole and finally, removal of the merozoite coat and some adhesins to allow parasite entry (Cowman and Crabb, 2006).

Selective capture of a host cell entails the interaction of receptors on the merozoite surface with ligands on the erythrocyte membrane. The identities of these have been difficult to establish, as the parasite can deploy multiple receptors able to engage with different host cell ligands, depending on parasite strain and erythrocyte genetics, thus maximizing the chances of invasion in genetically variant (polymorphic) hosts (Baum *et al*., 2005). Several large
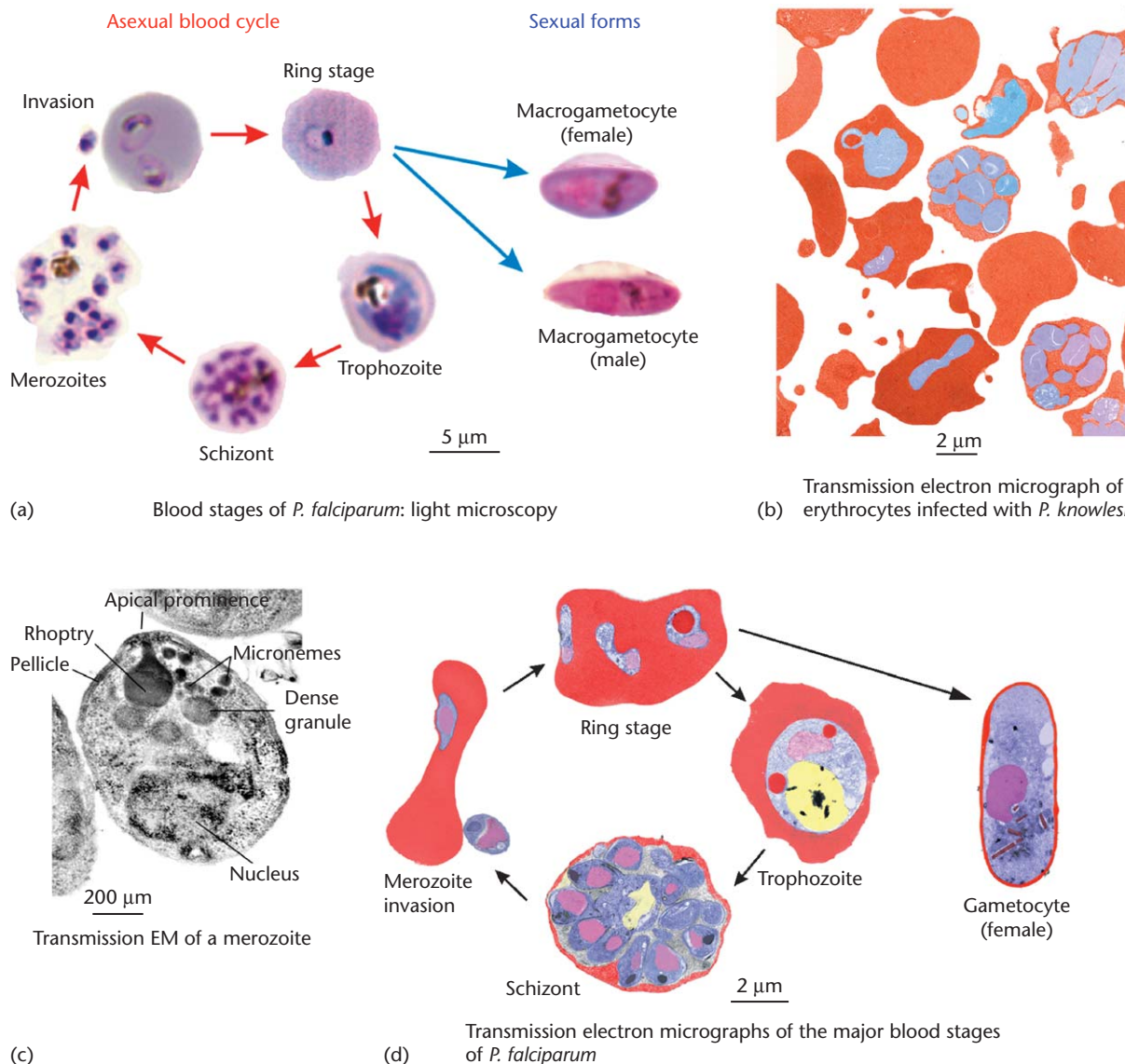
Asexual blood cycle   Sexual forms

(a) Blood stages of *P. falciparum*: light microscopy

(b) Transmission electron micrograph of erythrocytes infected with *P. knowlesi*

(c) Transmission EM of a merozoite

(d) Transmission electron micrographs of the major blood stages of *P. falciparum*

Figure 2 The stages of *Plasmodium falciparum* in the blood. (a) Light micrographs of infected erythrocytes stained as a blood film with Giemsa's stain are assembled into the major stages of asexual blood cycle and the sexual blood stages. (b) A transmission electron micrograph of a section through a blood sample infected with the simian malaria parasite *Plasmodium knowlesi*. A number of different stages are visible. To aid interpretation, false colour has been added to the monochrome micrographs, the parasites being coloured blue and the erythrocytes red. The same convention is followed in most other figures in this article. (c) An electron micrograph (EM) of a malaria merozoite, showing its main structural features. (d) Electron micrographs of the main blood stages of *Plasmodium falciparum* are assembled, coloured as in (b); nuclei are indicated in purple. Light micrographs of cells shown in (a) were provided by Gabriele Margos, Bath University, UK.

families of adhesive protein genes can be expressed alternatively in different merozoites. Most of them are secreted from micronemes, for example the erythrocyte-binding antigen (EBA)-175 (Adams *et al.*, 1992) and merozoite thrombospondin-like adhesive protein (MTRAP) whereas on the host erythrocyte side of attachment, glycophorins (sialylated glycoproteins) are important, though not essential ligands (for review see Cowman and Crabb, 2006). In *P. vivax*, the crucial erythrocyte ligand is the Duffy blood group antigen which binds the micronemal Duffy-binding protein (DBP, see Chitnis and Sharma, 2008). Many people with genetic roots in West Africa lack the Duffy blood group and are not infected with this species (innate immunity) (Miller *et al.*, 1975), although fully susceptible to *P. falciparum*. Another secreted micronemal protein is the apical merozoite antigen (AMA)-1 which is involved in the formation of the apical junction. Merozoite coat proteins also appear important in binding to erythrocytes, such as the Merozoite surface protein 1 (MSP-1) (Holder, 1994). Several of these proteins are at present being tested as
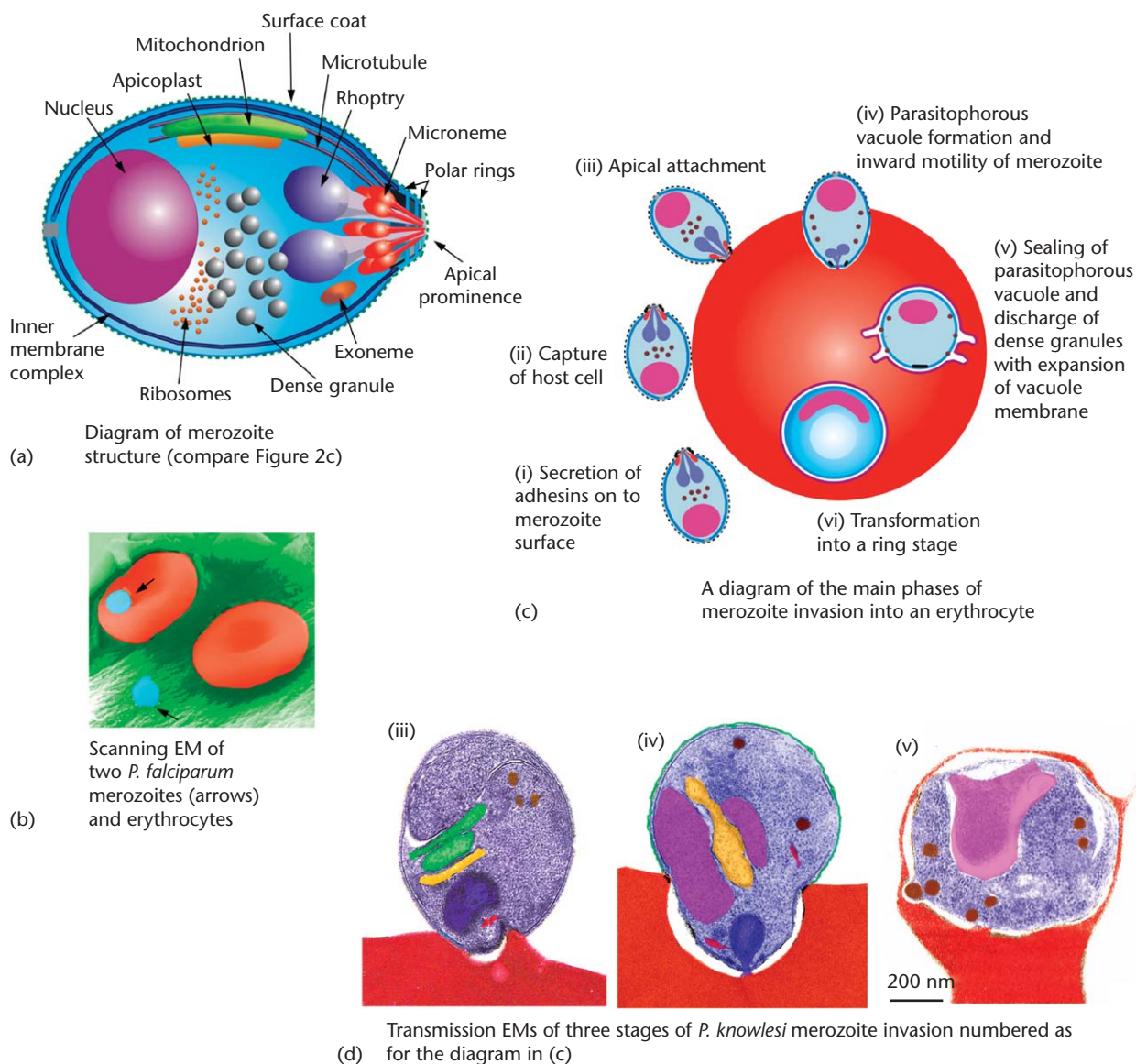
(a) Diagram of merozoite structure (compare Figure 2c)

(b) Scanning EM of two *P. falciparum* merozoites (arrows) and erythrocytes

(c) A diagram of the main phases of merozoite invasion into an erythrocyte

(d) Transmission EMs of three stages of *P. knowlesi* merozoite invasion numbered as for the diagram in (c)

Figure 3 (a)–(d) The merozoite and invasion. (a) A diagram shows the main organelles typical of *Plasmodium* merozoites. (b) Two merozoites of *Plasmodium falciparum* (arrows) and two erythrocytes have been imaged by scanning electron microscopy. (c) Depicts the main steps in merozoite invasion of an erythrocyte, related in (d) to three transmission EMs of invading *Plasmodium knowlesi* merozoites; false colours include green for a mitochondrion and orange for an apicoplast. (d (iv)) is altered from Bannister LH, Mitchell GH, Butcher GA and Dennis ED (1986). Lamellar membranes associated with rhoptries in erythrocytic merozoites of *Plasmodium knowlesi*: a clue to the mechanism of invasion. *Parasitology* **92**: 291–303, with permission of Cambridge University Press.

immunogens for vaccination against malaria. **See also**: Erythrocyte Membrane Disorders; Erythrocytes; Immunity to Infection; Infectious Diseases: Predisposition; Malaria: Immunity; Malarial Resistance and Susceptibility, Genetics of; Protein Families: Evolution; Vaccination of Humans

Intracellular Asexual Blood Parasite Stages

Ring and trophozoite stages: After entry, the merozoite loses its invasive organelles. It becomes disc- or cup-like with a thinner centre which under the light microscope or in some sections looks like a hole – hence the term 'ring' stage. It now begins to ingest erythrocyte cytoplasm, degrading the haemoglobin proteolytically (Goldberg, 2005) within small vacuoles (**Figure 2d**). As feeding progresses, the parasite transforms into a plumper trophozoite, and feeds more voraciously. A breakdown product of haemoglobin (haem) is converted to insoluble malaria pigment (haemozoin) in food vacuoles (**Figure 4a**, **e** and **f**). In *P. falciparum*, these coalesce into a single large central pigment-containing food vacuole (**Figure 2a** and **Figure 4a**).
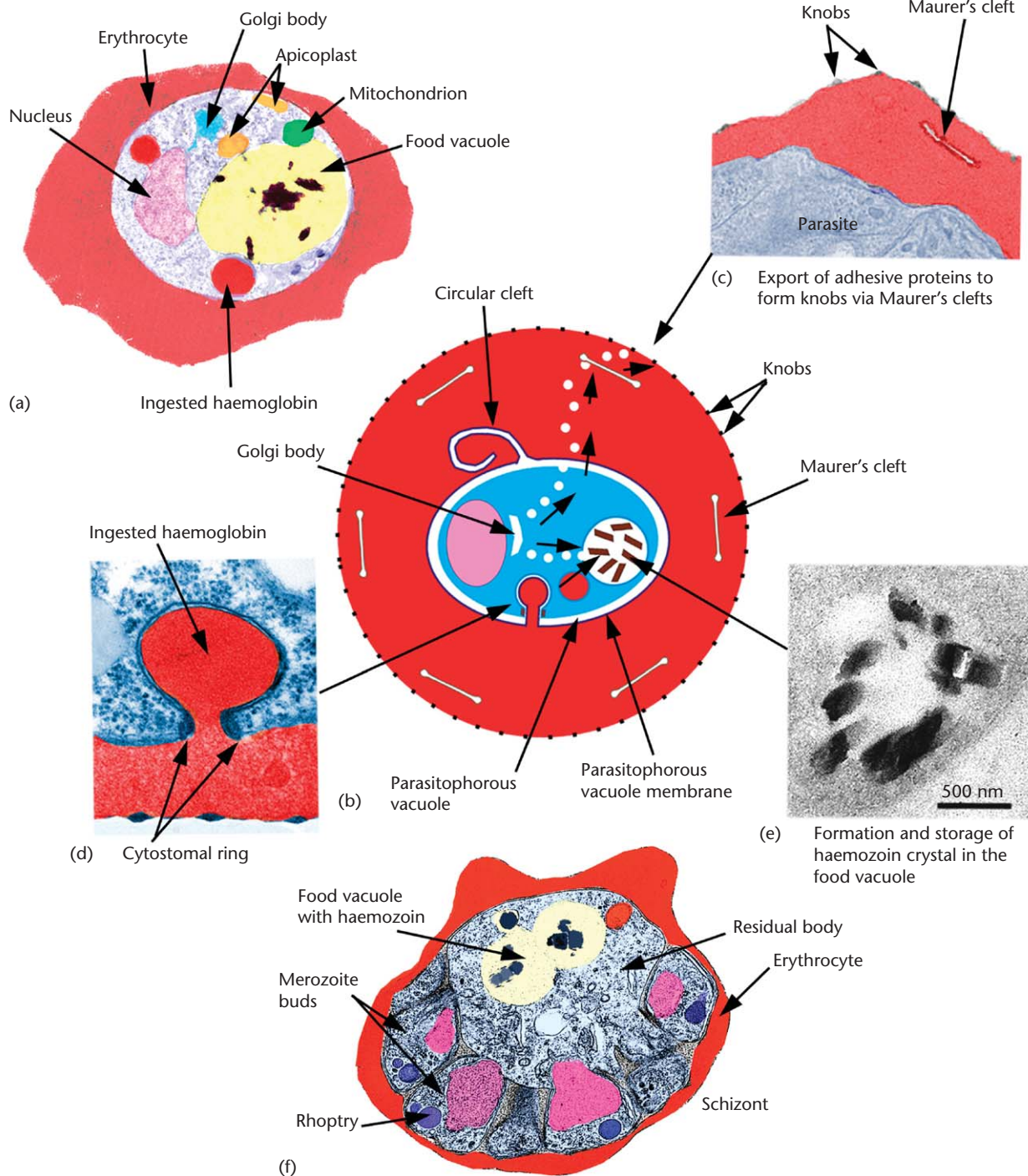
Figure 4 (a)–(f) Details of trophozoite and schizont organization. (a) shows major features of a trophozoite stage of *P. falciparum* imaged by transmission EM. The diagram (b) illustrates the mechanism of haemoglobin uptake, digestion and storage in a trophozoite, related in other panels to electron micrographs of (c) the export of adhesive proteins and knobs to the erythrocyte surface via Maurer's clefts, (d) ingestion of haemoglobin through the cytostome, and (e) formation of the malaria pigment haemozoin, which is left uncoloured to show the high density of individual pigment crystals. (f) shows an EM of a schizont during the process of merozoite budding from the main parasite mass (residual body).

Parasite-induced erythrocyte changes

As the parasite feeds it uses up the erythrocyte haemoglobin and at the same time exports parasite proteins which transform the erythrocyte surface. The first is the ring-expressed surface antigen (RESA) secreted from the merozoite's dense granules, which increases its stiffness and prevents invasion by other merozoites (Pei *et al*., 2007). Later, other proteins make the erythrocyte membrane more permeable to nutrients in the surrounding blood plasma. In some species (including *P. falciparum*) adhesive proteins are exported to the surface of erythrocytes, causing them to stick to blood vessel walls (sequestration) in the viscera and brain, preventing parasite removal by the spleen. Other exported proteins form small surface knobs which aid adhesion (**Figure 4c**). Some *P. falciparum* strains also adhere to placental blood vessel walls during pregnancy. Exported proteins can make infected erythrocytes adhere loosely to clusters of uninfected ones (rosetting). These different adhesive interactions cause severe pathology or death if large numbers of parasites block blood vessels, for example, in cerebral malaria, a common cause of death in *P. falciparum* infections. In pregnancy restriction of placental blood flow can also interfere with fetal development (Rowe and Kyes, 2004). In this species the erythrocyte membrane protein 1 (PfEMP-1) is a major adhesive receptor placed by the parasite on the infected erythrocyte surface, encoded by a large family of variant (*var*) genes expressed one at a time to bind various vessel wall ligands including in the placenta, chondroitin sulphate-A. This variability is thought likely to minimize its exposure to immune attack.

Export of parasite proteins to the erythrocyte surface is a complex process entailing (in *P. falciparum*) shuttling of small vesicles from the parasitophorous vacuole via flat membrane-lined cavities called Maurer's clefts, anchored to the underside of the erythrocyte membrane (**Figure 4b** and **c**), although some proteins move independently of vesicles to their targets (Lanzer *et al*., 2006). Other species have similar structures though with different details: for example *P. vivax* has surface indentations (caveolae) rather than knobs.

Schizont stage: Eventually the parasite's nucleus divides, repetitively, the final number depending on species (*P. falciparum* usually finishes with approximately 16, the result of 4 rounds of deoxyribonucleic acid (DNA) synthesis and mitosis). Each nucleus enters a merozoite bud (**Figure 2a** and **d** and **Figure 4f**) and other organelles are assembled near it. The mature merozoites detach from the parent schizont leaving a small amount of cytoplasm, the residual body, containing the haemozoin. Finally, protease released from specialized secretory vesicles (exonemes, **Figure 3a**) triggers a complex series of chemical changes causing merozoites to break out from the surrounding erythrocyte (Yeoh *et al*., 2007).

Feeding during the asexual blood cycle: The parasite ingests host cell cytoplasm (consisting mainly of haemoglobin) through specialized ring-like feeding structures, cytostomes (**Figure 4b** and **d**), into small vacuoles where a number of enzymes break down haemoglobin into the globin and haem components (Goldberg, 2005). The globin is further degraded by proteases to amino acids, some used by the parasite and the rest excreted. The iron-containing haem portion, ferroprotoporphyrin IX, is toxic but made safe by crystallization into the metabolically inert malaria pigment, haemozoin. Crystals of this yellow-brown pigment accumulate in food vacuoles (**Figure 4a** and **e**). However, the parasite consumes far more haemoglobin than it needs, a factor thought to prevent erythrocytes bursting due to osmotic influx of water because of the increased permeability of the erythrocyte membrane (see Mauritz *et al*., 2009). Some potent antimalarial drugs such as chloroquine inhibit haemozoin crystal formation, allowing accumulation of toxic haem which kills the parasite. However, some resistant parasite strains can prevent this inhibition, and resistance to chloroquine and similar drugs has become a major therapeutic problem globally. **See also**: Antiprotozoan Drugs; Protozoan Nutrition and Metabolism

Sexual Stages

Gametocytes: The reason why parasites switch to a sexual phase is not yet understood, but it occurs at or before the merozoite stage. Parasites develop into either male or female lines with their own sets of characteristic structure and chemistry, although their chromosomal contents are identical. In *P. falciparum* mature male and female gametocytes are long, often curved forms (**Figure 2a** and **d**) (the species name *falciparum*, sickle-bearer, refers to this shape), although they are spherical in many other species. The two genders differ from each other in cellular detail. During development both contain many long microtubules defining their shape, which varies with species, most being spheroidal. All mature gametocytes contain unique disc-like secretory vesicles (osmiophilic bodies) used in exit from the host cell, and are surrounded by a three-membrane pellicle (Khan, 2005; Alano, 2007).

Gametes: When gametocytes are ingested by the mosquito, they escape from their surrounding erythrocyte membranes, triggered by the temperature drop and by chemicals in the insect (e.g. xanthurenic acid; Billker *et al*., 1998). The male gametocyte divides rapidly into several gametes which sprout long motile flagella (exflagellation), enabling them to make contact with female gametes to fertilize them. **See also**: Protozoan Sexuality

Mosquito Asexual Stages

Ookinete: The zygote formed by fusion undergoes meiotic nuclear division and then elongates into a motile ookinete, developing large numbers of micronemes and polar rings though no rhoptries at its front end, and an inner membrane complex bearing myosin (**Figure 5a** and **b**). Using

Mosquito stages

Ookinetes

Apex — Nucleus

Micronemes

1 µm

(a)　　　· Light microscopy　　　(b)　　　Transmission EM

Sporozoites

10 µm

(c)　　(d)　　(e)　(f)

Apicoplast — Microtubule — Nucleus — Golgi body — Polar rings

Mitochondrion — Inner membrane complex — Rhoptries — Micronemes

1 µm

Anterior

(g)

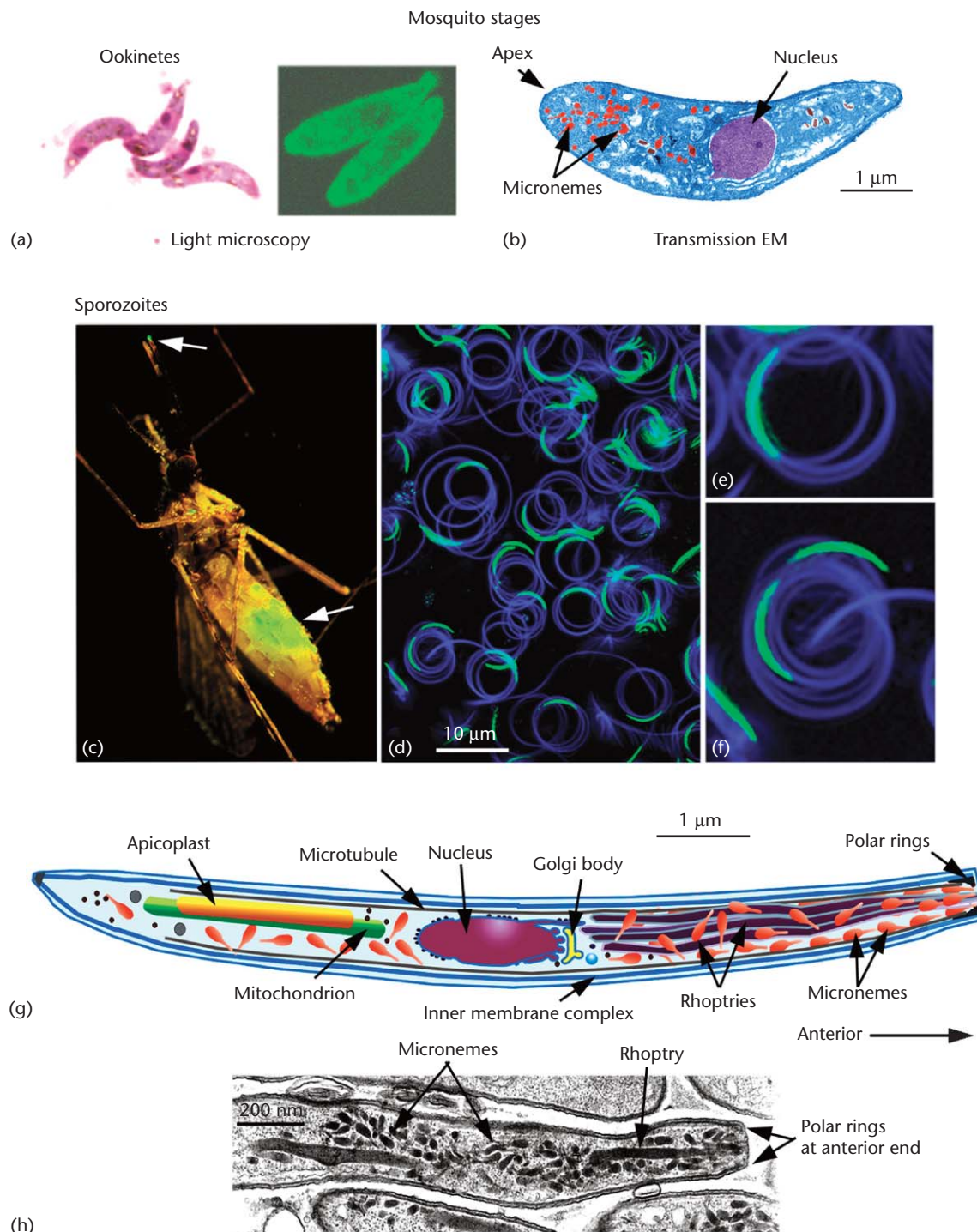Micronemes — Rhoptry — Polar rings at anterior end

200 nm

(h)

Figure 5 (a)–(h) Illustrations of *Plasmodium* mosquito stages. (a) and (b) show ookinetes of *Plasmodium berghei*, (a) by light microscopy (Giemsa-stained specimen, left and immuno-fluorescently stained for myosin, right), and (b) by transmission EM showing numerous micronemes, here coloured red, in the anterior region. (c)–(f) show sporozoites of *P. berghei* expressing green fluorescent protein after transfection, enabling clear visualization by fluorescence microscopy. (c) a female mosquito containing oocysts in its gut wall shows the presence of parasites by green fluorescence in its abdomen (lower arrow) and also some sporozoites in a drop of saliva at the tip of its proboscis (upper arrow); (d)–(f) show the movement of sporozoites gliding in circular trajectories on a glass surface, imaged to trace the direction of gliding, seen in more detail in (e) and (f). The diagram in (g) shows the major structures visible in a *Plasmodium* sporozoite, with its anterior end towards the right. For comparison, an EM of the anterior part of a *P. berghei* sporozoite is shown in H, showing the elongated rhoptries and numerous micronemes crowded in this region. Original images shown in (a) were provided by Dr Inga Siden-Kiamos and (b) Dr Anton Dluzewski, Institute of Molecular Biology and Biotechnology, Heraklion, Crete. (c), (d), (e), and (f) were provided by Dr Sylvia Münter and Dr Friedrich Frischknecht, University of Heidelberg Medical School, Heidelberg, Germany. (h) is reproduced by permission of Cambridge University Press from Schrével *et al.* (2007).

secreted enzymes, the ookinete penetrates the gut epithelium before rounding up and secreting a cyst wall, a stage called the oocyst (**Figure 1**).

The oocyst and sporogony: Within the oocyst the parasite replicates its DNA many times to form a massive nucleus containing hundreds of genomic centres by repeated internal mitotic divisions. The groups of chromosomes separate into individual nuclei around the parasite periphery, and move into finger-like projections at the parasite surface, each of these generating new organelles and eventually detaching as a sporozoite. When mature these migrate through the cyst wall into the insect blood cavity (haemocoel).

Sporozoites: These are much longer than merozoites, although built to a similar structural plan (Kappe *et al.*, 2003; **Figure 5c–h**). They are elongate slightly curved cells (in *P. falciparum*) approximately 10 μm long, tapering at both ends, with a central nucleus, apical organelles (polar rings, a set of rhoptries – 4 in *P. berghei* (Schrével *et al.*, 2007) and numerous micronemes) (**Figure 5g** and **h**). To the inner membrane complex around its periphery is attached a set of longitudinal subpellicular microtubules stretching back from the polar rings to create a spiral cage-like cytoskeleton around the parasite (Cyrklaff *et al.*, 2007). A single mitochondrion and an apicoplast lie posteriorly. Sporozoites glide in shallow curves on flat surfaces (**Figure 5c–f**) and can negotiate the three-dimensional tangles of fibres in the host's skin as well as liver cells after infection by mosquito bite (Amino *et al.*, 2006). Like the merozoite, its apical organelles contain a battery of invasive proteins – adhesins such as the circumsporozoite protein (CSP), and thrombospondin-related antigen protein (TRAP) which enable it to attach to the glycosaminoglycans of its target host cells. These are important candidate immunogens for current human antimalaria vaccine development (Sauerwein, 2009).

Pre-erythrocytic Stages

To reach a suitable liver cell (hepatocyte), a sporozote in the bloodstream penetrates defensive hepatic macrophages (Kupffer cells), the vessel wall lining (endothelium) and may traverse several hepatocytes before entering its final hepatocyte home (Frevert, 2004; Frevert *et al.*, 2005; Meis *et al.*, 1990; Sturm *et al.*, 2009). In the hepatocyte the parasite feeds, grows and multiplies (as pre-erythrocytic trophozoites and schizonts) to generate many hundreds of merozoites. Eventually clusters of these are shed into the bloodstream inside membrane-lined packets (merosomes). These pass into the pulmonary circulation and impact in the small alveolar vessels, where the merosome membrane breaks down to release the merozoites into the general circulation (Baer *et al.*, 2007). The pre-erythrocytic phase lasts from 2 days to 3 weeks, depending on species. In *P. falciparum* it is approximately 9 days, but in *P. vivax* and some other malarias liver stage parasites can lie dormant for months or years (the hypnozoite stage) before

finally maturing to release merozoites into the bloodstream long after initial infection (a relapse). **See also**: Malaria

Metabolism

During the life cycle there are constant changes in the nature of the parasite's metabolism (Olszewski *et al.*, 2009). In asexual blood stages, proteolysis of haemoglobin provides most amino acids for parasite growth, but methionine, arginine and isoleucine from the blood plasma are also required (Olszewski *et al.*, 2009). Blood glucose, the principal energy source, is metabolized anaerobically to lactic acid; aerobic metabolism does not serve for energy production – instead the electron transport pathway is linked to the *de novo* biosynthesis of pyrimidines (thymidine and cytidine). In contrast, because the parasite is unable to synthesize purines (adenosine and inosine) *de novo* it must rely on salvage mechanisms. Hypoxanthine is probably the primary source for these purines. Although *Plasmodium* is capable of fatty acid synthesis, it largely relies on external sources (erythrocyte and plasma) for lipid biosynthesis. However, type II fatty acid synthesis, a feature of the apicoplast, appears to be essential for liver stage development (Yu *et al.*, 2008). **See also**: Fatty Acid Synthesis in Protozoan Parasites; Protozoan Nutrition and Metabolism

The *Plasmodium* Genome

The genome is carried on a single set of chromosomes, 14 in number in all species studied, bearing in *P. falciparum* 23 million base pairs (Gardner *et al.*, 2002) and encoding at least 6000 genes, although the complexities of gene expression predict a larger number of proteins. Every species of *Plasmodium* studied has a single set of chromosomes (i.e. the haploid number) in all stages except immediately after fertilization in the mosquito, where meiosis and the exchange of genes between homologous chromosomes occur in a classical Mendelian manner (Walliker, 2005). In all other stages multiplication involves mitosis, which occurs as in many unicellular organisms without the total breakdown of the nuclear membrane (endomitosis).

Gene expression varies in distinctive time-dependent patterns in the different phases of the life cycle (Bozdech *et al.*, 2003). Although protein synthesis is broadly typical of eukaryotes, there are some unusual features; in some species including *P. falciparum* the DNA is unusually rich in adenosine and thymine bases, and transcription regulation also appears to be atypical (Yuda *et al.*, 2009). As in some other parasitic protozoans, there are many polymorphic genes and several instances of large variant gene families, strategies which hinder the recognition of parasite proteins by the host's immune system when proteins expressed at surfaces are exposed to antibody attack. **See also**: Genome Databases; Malaria: Immunity; Malarial Resistance and Susceptibility, Genetics of

Proteins destined for export are synthesised in the endoplasmic reticulum which is continuous with the nuclear envelope and then passed to the Golgi body (see Bannister *et al.*, 2004; Struck *et al.*, 2005). From this, vesicles are issued either for immediate export, as in ring and trophozoite stages, or to create the secretory organelles and other membranous organelles of invasive stages. The mitochondrion and apicoplast each contain their own DNA and ribosomes encoding some of their own proteins, though the majority of their genes are in the nucleus. Unlike most eukaryotes, very few proteins are glyocosylated, apart from the glycosylphosphatidyinositol (GPI) moiety that anchors several surface proteins of infective stages (see von Itzstein *et al.*, 2008).

Motility

All the invasive stages are motile, either during intracellular invasion (merozoites, sporozoites) or when gliding on surfaces (ookinetes, sporozoites, see **Figure 5c**–**f**). In all cases motility depends on molecules of nonmuscle myosin (type XIV) anchored to the inner membrane complex of the pellicle, with actin filaments. It is envisaged that actin becomes attached through transmembrane links to adhesive molecules on the parasite surface which in turn grip the surface of a host cell adjacent to the parasite. Propulsion of actin filaments by myosin then causes the parasite to glide forward (see for recent review Matuschewski and Schüler, 2008).

Recent History of *Plasmodium* Research

The geographical association of malaria with marshy environments was recognized even in antiquity, although it was only towards the end of the nineteenth century that *Plasmodium* was recognized in infected blood and its transmission by mosquitoes established by Laveran, Golgi, Ross, Grassi and others. Approximately 50 years later the pre-erythrocytic stages in the liver were shown by Garnham and Shortt. Since then many discoveries have defined parasite classification, structure, molecular composition, biochemistry, physiology, genetics and immunology. These advances have depended on the growth of molecular and computer technologies, and the discovery of how to culture *P. falciparum in vitro* (Trager and Jensen, 1976; Haynes *et al.*, 1976). A major recent landmark of much importance for present and future malaria research is the complete sequencing of several *Plasmodium* genomes, first of *P. falciparum* (Gardner *et al.*, 2002), and later *P. vivax*, *P. knowlesi*, *P. berghei*, *P. chabaudi* and *P. yoelii* (see the PlasmoDB database; Aurrecoechea *et al.*, 2009). These developments have led to a rapid expansion of research into the structure and functions of proteins expressed through the life cycle, using novel techniques of genetic modification and high throughput expression analysis which are transforming our view of this genus, and also providing a wealth of data for possible new drug and vaccine targets (see Hall *et al.*, 2005; Sauerwein, 2009). For a detailed account of historical aspects. **See also**: Garnham, Percy Cyril Claude; Grassi, Giovanni Battista; High Throughput "On Chip" Protein and Nucleic AcidTransfection; History of Antimalarial Agents; History of Malaria; Ross, Ronald; Shortt, Henry Edward; Trager, William

Evolution of *Plasmodium*

Current estimates suggest that now some hundreds of *Plasmodium* species exist, some recognized by classical parasitology, but many others suggested from molecular studies (see Martinsen *et al.*, 2008). They appear to have diverged into two groups early in their history – those infecting mammals and those with bird or reptile hosts. Genomic analysis shows that *P. falciparum* and the chimpanzee parasite *Plasmodium reichenowi* form a distinctive subgroup within the mammalian division but may have split off early, leaving the main group to diverge later into rodent and other primate parasites. Using data from comparative genomics ('molecular clock'), evidence indicates that the *P. falciparum*–*P. reichenowi* divergence occurred 6–8 million years ago, when the human and chimpanzee evolutionary lines also diverged. Interestingly DNA studies show that another genus, *Hepatocystis*, which infects bats, belongs to the mammalian group of malarias (Martinsen *et al.*, 2008). Genomic data suggest that *P. vivax* arose by lateral transfer from monkeys to humans in Asia, then spread to Africa, and separately by human migration via the Alaskan route to the Americas where lateral transfer in the opposite direction, to monkeys appears to have occurred (Cornejo and Escalante, 2006), *P. simium* being genetically identical. Similarly *P. malariae* appears to have transferred to monkeys there as *P. brasilianum*. **See also**: Molecular Clocks; Molecular Evolution

Conclusion

The complex life history and subtle mechanisms for parasitizing two sets of hosts have enabled the species of *Plasmodium* to exploit a range of cells and tissues in many vertebrate species over many millions of years. Indeed the evolution of the human race is intricately entangled with malaria, seen clearly in human genetic variation, sometimes otherwise deleterious but giving a measure of protection against malaria. Examples include some haemoglobinopathies such as sickle cell anaemia, the thalassaemias, and haemoglobin C; a range of blood group variants (e.g. Duffy-negative, Group O, etc.), glucose-6-P dehydrogenase (G6PD) deficiency, hereditary ovalocytosis, immune system polymorphisms (Kwiatkowski, 2005), and many others that have shaped the history of the human race, with which *Plasmodium* continues to be entwined. **See also**: Balancing Selection in Human Evolution; Blood Group Genetics; Glucose-6-Phosphate

Dehydrogenase (G6PD) Deficiency: Genetics; Malaria: Immunity; Malarial Resistance and Susceptibility, Genetics of; Selection and Common Monogenic Disease; Sickle Cell Anaemia; Susceptibility to Malaria, Genetics of

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