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Molecular machinery of signal transduction and cell cycle regulation in *Plasmodium*

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Abstract

The regulation of the *Plasmodium* cell cycle is not understood. Although the *Plasmodium falciparum* genome is completely sequenced, about 60% of the predicted proteins share little or no sequence similarity with other eukaryotes. This feature impairs the identification of important proteins participating in the regulation of the cell cycle. There are several open questions that concern cell cycle progression in malaria parasites, including the mechanism by which multiple nuclear divisions is controlled and how the cell cycle is managed in all phases of their complex life cycle. Cell cycle synchrony of the parasite population within the host, as well as the circadian rhythm of proliferation, are striking features of some *Plasmodium* species, the molecular basis of which remains to be elucidated. In this review we discuss the role of indole-related molecules as signals that modulate the cell cycle in *Plasmodium* and other eukaryotes, and we also consider the possible role of kinases in the signal transduction and in the responses it triggers.

Keywords

Plasmodium; Malaria; Serpentine receptor; Calcium; Kinases

1. Introduction

The molecular mechanisms responsible for eukaryotic cell cycle control are likely to have been highly conserved during evolution. There is indeed evidence that fundamental principles are conserved between yeast and mammals [2]. However, yeast and metazoans are both members of the same phylum (*Opisthokont*) [3] and much more detailed analysis in different eukaryotic groups is needed before a full picture can be established. In addition to its importance in fundamental biology, research into cell cycle control carries a strong potential for application in drug discovery. Many elements of the cell cycle control machinery are important targets for the development of new drugs against cancer and other pathologies. The many unusual features of eukaryotic pathogens (including *Plasmodium* spp.) suggest that cell cycle control could be selectively targeted to create a new range of anti-parasitic drugs.

Plasmodium has a complex life cycle alternating between two hosts (1) mosquitoes, in which sexual reproduction occurs, and (2) a vertebrate, where *Plasmodium* invades and multiplies asexually in erythrocytes and hepatocytes. The parasite undergoes major metabolic and

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morphological changes as it exploits its two hosts, with periods of intensive cell division as it multiplies during sporozoite formation (sporogony) in the mosquito gut wall, and in the liver and red blood cells (schizogony) of the vertebrate host.

The intraerythrocytic phase is the cause of malaria pathogenesis. This phase consists of cycles of invasion, multiplication and reinfection. It begins with the invasion of erythrocytes by merozoites and continues into growth (ring and trophozoite stages), formation of multiple new merozoites (schizont stage) and finally release into the bloodstream of merozoites that in turn will infect new erythrocytes.

In *Plasmodium falciparum*, the most virulent of the four *Plasmodium* species that infect humans, and in the rodent malaria parasite *P. chabaudi*, the erythrocytic cycle events usually occur synchronously during *in vivo* infection but synchrony is lost in *in vitro* cultures, presumably because some defining factor present in the host is absent from the culture medium. There are artificial ways of restoring synchrony of *P. falciparum in vitro*, such as temperature elevation [4] or the addition of sorbitol [5] which, however is not related to normal control since it entails the killing of all but ring stages. Of greater biological significance is the reported modulation of synchrony of *P. falciparum* by host tryptophan-derived molecules [6].

The cell cycle comprises a range of highly ordered events that lead to mitosis and the formation of new cells. Regulation of these conserved processes is critical to normal cellular growth, differentiation and replication. According to Hammarton et al.[7] merozoites and rings are in G1, and the S phase begins when the parasite is at trophozoite stage, while in schizont stage, merozoites are produced by successive rounds of poorly understood mitosis [1]. To regulate these processes cells employ several mechanisms including phosphorylation, transcription control and degradation of regulatory proteins by the proteasome complex.

2. Receptors: the upstream part of signaling pathways

In many instances the sensing of environmental cues and ensuing signal transduction are crucial to initiate the cell cycle and/or cell differentiation [8]. These occur when a stimulus binds to a receptor and promotes downstream responses such as transcription regulation, alterations in metabolism, cell proliferation and apoptosis, all mediate by second messengers and effectors. Cells may sense a range of stimuli such as hormones, light, growth factors, cytokines and other molecules, and each cell type may express its own repertoire of receptors detecting specific signals. The more spread family of receptor is GPCR (G-protein coupled receptors) which are involves in a range of physiological processes.

Many organisms use external molecular signals to drive cell differentiation. Examples include several species of amoebae, which modulate encystation by sensing hormones such as catecholamines by means of transmembrane receptors [9], and fungi that secrete molecules inducing cell differentiation [10]. Indole is an important signaling molecule not only in eukaryotes, but also in bacteria, where it has been implicated in quorum sensing processes [11] and in the cell cycle control of *Escherichia coli* [12].

Within cells, second messengers like calcium and cAMP are able to promote a range of intracellular responses. An increase in cytosolic calcium concentration is caused by the release of calcium from intracellular pools such as the endoplasmic reticulum [13], mitochondria [14] and acidocalcisomes [15,16] and also by influx through the plasma membrane. In *Plasmodium* there are several reports from different labs implicating calcium signaling at several stages of the life cycle, including erythrocytic schizogony, gametogenesis, ookinete motility, [17,18]. Moreover increase of intracellular second messengers concentration as cAMP and calcium are involved in a range of signaling events in *Plasmodium* signaling such as in tryptophan-derived response [19,20] and in sporozoite apical regulated exocytosis [21].

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cAMP also inhibits maturation of merozoites in RBCs [22] and in is able to promote sexual differentiation [23–25].

Since Martin et al. [26] reported that gametocytes of *P. falciparum* produce $InsP_3$ during exflagellation it is possible that calcium increase via PLC could be caused by $InsP_3$. Furthermore Passos and Garcia [27] promote *in vitro* calcium increase by adding $InsP_3$ in *P. falciparum* culture.

Another second messenger, cGMP and subsequently PKG activation together with calcium release are crucial for xanthurenic acid-induced gametogenesis into mosquito [28].

As already mentioned, synchrony of erythrocytic schizogony in some *Plasmodium* species occurs *in vivo* and is lost *in vitro*. In *P. falciparum*, treatment of trophozoites with melatonin activates a calcium/cAMP-dependent response that, at least *in vitro*, is able to synchronize their intraerythrocytic stages [29]. Other tryptophan-derivatives such as tryptamine, N-acetylserotonin, and serotonin also promote changes in the *Plasmodium* cell cycle [20,30]. N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), a product of melatonin degradation, is able to synchronize *P. chabaudi* and *P. falciparum* proliferation [31].

Furthermore, in *P. chabaudi*, erythrocyte rupture and reinvasion occur approximately between midnight and 3 a.m., which coincides with the circulating melatonin peak level. When *P. chabaudi* infects pinealectomized mice, which lack melatonin, synchrony is lost but melatonin administration is able to restore it; this effect of melatonin is inhibited by the addition of luzindole (a melatonin antagonist) [19]. Administration of a suboptimal concentration of the anti-malarial drug, chloroquine in addition to luzindole reduces significantly the mortality of mice infected with *P. chabaudi* [32].

Calcium release caused by melatonin treatment also activates several cysteine-proteases acting in erythrocyte rupture, hemoglobin and cytoskeletal proteins degradation [33]. Interestingly, it has been found in the laboratory of one of the authors that in contrast to *P. chabaudi* and *P. falciparum*, asynchronous *P. berghei* is insensitive to melatonin with respect to both [Ca^{2+}] increase and cell cycle modulation [34].

In vertebrates, melatonin receptors belong to the family of G-protein coupled receptors (GPCRs), also called seven transmembrane (7TM) receptors [35]. This family is widespread in eukaryotes. To mention just two examples, *Dictyostelium discoideum* uses GPCR signaling for many cellular processes including development [36], and the parasitic helminth *Schistosoma mansoni* uses a GPCR to sense histamine and to trigger cell responses via calcium and cAMP [37].

Madeira et al. [38] identified four putative serpentine receptors in *P. falciparum* and their functions are under analysis. Their predicted roles in sensing extracellular signals, possibly in the form of host hormones or other molecules, make these four putative receptors potentially very interesting, and elucidation of their detailed function may shed light on how the parasite modulates its life cycle in response to its environment Fig. 1 depicts schematic model for molecular signaling machineries in *Plasmodium.*

It has been proposed that hormones might influence malarial infection, for example in pregnant women infected with *P. falciparum* [39].

Although trimeric G-proteins are not found in *Plasmodium* genome, experiments with cholera and pertussis toxin brought evidences about its expression at the intraerythrocytic stage [25]. The inhibition of Gαs protein with peptides diminished *P. berghei* parasitemia [40]. On the

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other hand it is reported that Gαs present in erythrocytes is recruited to the malarial vacuole [41].

The knowledge of the downstream mechanisms involved in signal transduction pathways in *Plasmodium* is fundamental to understand parasite biology. Protein kinases (PKs) are an important family of proteins that are expected to regulate diverse cellular activities. In the sections below we present a brief overview of current knowledge on *Plasmodium* kinases.

3. Protein kinases: signal transducers

The *P. falciparum* genome sequence contains 85 or 99 protein kinases (PK)-related sequences, depending on the study [42,43]. The number is rather low in comparison to the size of the kinome in *Saccharomyces cerevisiae*: while the yeast genome contains a similar number of total genes to that of *P. falciparum*, it possesses significantly more PKs. This low number might be attributed to the intracellular lifestyle of the parasite, which is likely to receive less environmental cues than a free-living unicellular eukaryote. However, complex life cycle stages of malaria parasites in mosquito and the vertebrate host entail intricate regulatory mechanisms where protein kinases are expected to be key molecules. Therefore, it is possible that malaria PKs may possess particularly complex cellular functions. Given that many *Plasmodium* PKs have atypical features compared to their eukaryotic homologues they are predicted to be promising targets for antimalarial development [42,44].

3.1. Calcium modulated protein kinases

Calcium-mediated intracellular signaling is increasingly being found to be important for an assortment of cellular function in apicomplexans, and calcium signaling has been implicated in the response of *Plasmodium* to melatonin [29,45]. A major class of downstream effector of Ca^{2+}-mediated signal transduction in *Plasmodium* is the CDPK family. These proteins have a conserved NH_2-terminal ser/thr protein kinase domain that is fused to a COOH-terminal calmodulin-like domain containing four EF-hand calcium-binding sites; proteins sharing a similar domain organization are found in plants and Alveolates, but not in metazoans. The majority of PfCDPK genes exhibit significant expression in the sexual stages [42]. Elegant reverse genetics work in *P. berghei* by Billker et al. showed that CDPK4 is a key enzyme in male gamete formation, regulating entry into S phase during gametocyte activation [17]. CDPK4 also exhibits a potential role in sporogonic development because there is a significant reduction of mosquito infectivity of ookinetes derived from ΔCDPK4 macrogametes [17]. The *Plasmodium* CDPK3 is exclusively expressed in the ookinetes [46]. Although *cdpk3* disrupted *P. berghei* lines exhibited normal exflagellation and development into ookinetes, their transmission efficiency is severely affected as evidenced by reduction of the number of oocyst in the midgut [46,47]. The reason for this drop in oocyst number was shown to be due to a defect in gliding motility [46,47]. CDPK6 is another CDPK that is dispensable for the asexual cycle; *P. berghei* parasites lacking CDPK6 are competent for sporozoite formation, but the sporozoites are significantly less infective for hepatocytes than wild-type parasites [48]. In contrast, PfCDPK1 is essential for erythrocytic schizogony [49] and is localized to parasite or parasitophorous vacuolar membrane [50], consistent with a proposed role in motility. The targeting of PfCDPK1 to these membranes was found to be dependent on the N-terminal dual acylation and basic residue motifs. Interestingly, Raf kinase inhibitor protein (RKIP) ortholog, which is also a protein kinase C (PKC) substrate in mammals, modulates PfCDPK1 activity *in vitro* [51]. Whether or not PfCDPK1 functions like PKC in *Plasmodium* remains to be established, but these results highlights atypical properties of *Plasmodium* kinases that cannot be discerned simply based on homology because many of these proteins are expected to possess parasite-specific functions. It is interesting to note that a receptor for activated protein kinase C (PfRack) has been identified in *Plasmodium* [52].

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The importance of Ca^{2+} in the intracellular signaling of malaria parasites is exemplified by the existence of a novel Ca^{2+}/calmodulin-regulated protein kinase B (PfPKB). Unlike the mammalian PKB, the PfPKB does not contain the N-terminal pleckstrin homology domain that interacts with phosphoinositides to regulate its activity. Instead this novel N-terminal region of PfPKB exhibits Ca^{2+}-dependent interaction with calmodulin for its activation [53]. Involvement of phospholipase C (PLC) in PfPKB activation was suggested as U73122, a specific inhibitor of phospholipase C, inhibited kinase activity [53]. Recently, it has been shown that treatment with a peptide inhibitor that competes with calmodulin binding to PKB and A443654, a small-molecule inhibitor of PKB, inhibits invasion of merozoites [54]. PfPKB was also shown to phosphorylate PfGAP45, a glideosome-associated protein, suggesting its role in invasion [54].

It has been also shown that the cGMP-dependent protein kinase (PKG) in *P. falciparum* may function upstream of events that mobilize Ca^{2+} and is likely to be a key regulator of gametogenesis [28]. While the anticoccidial PKG inhibitor compound 1 inhibits gametocyte rounding up and subsequent exflagellation, gametocytes in which inhibitor-insensitive PKG has been incorporated in the genome, through allelic replacement, rounds up normally [28].

It is evident that Ca^{2+} is an important second messenger that regulates various cellular processes in *Plasmodium* (Fig. 1). To elucidate the cellular response to Ca^{2+} signals in malaria parasites it will be important to understand the mechanism by which the Ca^{2+} level is regulated and the role of different Ca^{2+}-regulated proteins.

3.2. Cyclic nucleotide-dependent pathway

Cyclic nucleotide monophosphates, cAMP and cGMP are important second messengers in eykaryotic cell synthesized by adenylyl cyclases (PfAC) and guanylyl cyclases (PfGC), respectively. Malaria genome encodes two distinct PfACs [55]. PfACα contains six potential transmembrane domains at the N-terminus that have structural features of voltage-gated K^+ channel and a C-terminal adenylyl cyclase domain. The unique features of PfACα suggest that the changes in ion conductance may be coupled to cAMP synthesis. The PfACβ is related to a family of soluble ACs found in photosynthetic bacteria and humans [56]. It has been shown that cAMP may have a role in sexual differentiation of the parasite [24,57]. In eukaryotes, one of the major roles of cAMP synthesized by ACs is to activate cAMP-dependent protein kinase (PKA) by binding to the inhibitory regulatory subunit PKAr. *P. falciparum* PKA catalytic (PKAc) [58] and the regulatory subunits have been characterized [59]. Using patch-clamp technique in infected erythrocytes, it has been shown that either addition of PfPKAr or overexpression of PfPKAr in *trans* leads to down-regulation of host cell anion conductance. Furthermore, PfPKAr overexpressing line exhibits reduced growth, which could be corrected by increasing the intracellular cAMP level [59].

Two guanylyl cyclases, PfGCα and PfGCβ with catalytic activity have been identified in *P. falciparum* [60]. Interestingly, the PfGCs appear to be bifunctional as they also contain P-ATPase domain at the N-terminal extension [56,60]. The PfGCα gene is expressed in both asexual and sexual stages [61] and cannot be deleted [62] suggesting its essentiality. Although previous pharmacological studies suggested the role of cGMP in exflagellation, recently it has been shown that the disruption of PfGCβ has no effect on gametogenesis [62]. However, disruption of phosphodiesterase PfPDE) δgene affects gametogenesis. This suggests the importance of PfPDE in maintaining the level of cGMP during sexual development of the parasite.

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3.3. MAP kinase pathway

The mitogen-activated protein (MAP) kinases play a central role in coordinating activity of multiple intracellular mediators. *P. falciparum* genome encodes two homologues of MAP kinases [42], *pfmap-1* and *pfmap-2*, and both loci have been disrupted to understand their function. While *pfmap-1* knock-out lines do not have any phenotype in erythrocytic schizogony and sporogony, *pfmap-2* is essential for asexual growthand the loci can only be disrupted when an episomal copy of *pfmap-2* is present suggesting its essentiality [63]. Interestingly, the *P. berghei* homologue of PfMAP-2 was shown to be nonessential in asexual stages and gametocytes but is important for male gamete formation [64,65]. It is noteworthy that there are no unambiguous orthologues of MAP kinase kinase (MAPKK) or MEK in *P. falciparum*. PfPK7 appears to be a novel chimeric protein whose C-terminal region has identity with MEKs, whereas the N-terminal lobe shows homology to fungal protein kinase A [66]. Furthermore, PfPK7 does not contain the activation site in its T-loop and is insensitive to MEK and PKA inhibitors [67]. Recent determination of PfPK7 structure at 3.7Å resolution showed, however, that its structure is similar to TAO2 kinase, a MAP3KKK [68]. Although PfPK7 was not essential for the asexual growth, malaria parasites in which *pfpk7* locus was disrupted grows slowly with a reduced number of merozoites per segmenters compared to the wild type [66]. PfPK7 deficient parasite lines also have severe defects in oocysts production [66].

Recently, it has been suggested that PfNek3, one of the *P. falciparium* homologue of NIMA-like kinases that are involved in cell cycle regulation, particularly G2/M transition, in eukaryotes [69] activates PfMAP2 *in vitro* through phosphorylation, a feature that had previously been described for Pfnek-1 [70–72]. These results underscore unique function of *Plasmodium* kinases that will be difficult to perceive by homology analysis. NIMA-related kinases (NEKs) usually regulate cell cycle progression in eukaryotes [73]. *Plasmodium* genome encodes four homologues of these proteins that are expressed mainly in gametocytes [74]. Although PfNek4 is expressed in gametocytes, its disruption in the *P. berghei* has no influence in gamete formation or fertilization of gametes but differentiation of zygotes to ookinetes is interrupted [75]. PbNek4 was shown to be essential for the replication of diploid zygote genome before meiosis ensues.

3.4. CDK-like kinases and other putative cell cycle kinases

Although the developmental stages of malaria parasite are unique and complex, it is expected that proteins belonging to the CDK-related subfamily will be key regulators in the *Plasmodium* similar to eukaryotic cell cycle. Among the Pf protein kinases clustering within the CMGC group (to which CDKs and MAP kinases belong), PfPK5 clusters with CDK1/2 and Pfcrk-1 with CDK10/11. PfPK5 was the first CDK-like kinase characterized in *P. falciparum* with 60% identity to human CDK1 [76]. PfPK5 is expressed throughout erythrocytic schizogony, and immunoprecipitation experiments using synchronized parasite extracts showed that PfPK5 activity peaks at the schizont stage around 36 h post-invasion [77,78]. PfPK5 also colocalizes with the nuclear stain [78]. The structure of PfPK5 has been determined to a resolution of 1.9Å [79]. PfPK5 has structurally homology to human CDK2, the only other monomeric CDK structure solved.

PfPK6 is novel protein showing identity to both CDKs and MAP kinases by differential display RT-PCR of mRNA samples undergoing transition from ring to schizonts [80]. Molecular modeling data suggests that PfPK6 is more closely related to the CDKs [81,82] rather than MAP kinases. The PSTAIRE motif is replaced by a SKCILRE sequence in PfPK6, but the sites of regulatory phosphorylation are conserved. PfPK6 appears to be a novel cyclin-independent kinase. Another CDK-related kinase identified was Pfmrk, a homologue of the Mo15/CDK7 CDK-activating kinase [83]. Recombinant Pfmrk displays very little histone kinase activity as a monomer, but can be activated by the presence of human cyclin H and Pfcyclin-1 [84,85].

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Recently, it was shown that Pfmrk is activated by PfMAT1 homologue in presence of cyclin, similar to what is observed with CDK7/cyclinH-MAT complex in other eukaryotes [86]. Pfcrk-1, Pfcrk-3, and Pfcrk-5 are other CDK-like kinases [81]. Pfcrk-1 is not expected to be a functional homolog of eukaryotic CDK1/2; instead it belongs to the p58GTA gene family that is a negative regulator of cell growth [87]. Pfcrk-1 exhibits peak expression in gametocytes, but the *P. berghei* orthologue was shown to be essential for completion of the asexual cycle [88]. Four *P. falciparum* cyclin homologues, Pfcyc1–4, have been identified [84,89]. Pfcyc1 has maximum homology to the cyclin H family, an activator ofCDK7. As expected, Pfcyc1 activated Pfmrk (a putative CDK7 homologue) [85] but, surprisingly, Pfcyc-1 also activated PfPK5 [84]. Members of the cyclin H family are specific activators of CDK7 and not CDK1 or CDK5 (to which PfPK5 has the highest homology). PfPK5 has also been shown to be activated by mammalian cyclin A, p25 and RINGO [74,84,89]; such promiscuity for various cyclin-related proteins has not been reported for mammalian or yeast CDKs. Both p25 and RINGO are non-cyclin CDK activators from vertebrates. Three additional cyclins, Pfcyc2, Pfcyc3, and Pfcyc4 have been identified recently [90]. Pull-downand co-immunoprecipitation experiments showed that these cyclins associate with histone H1 kinase activity in parasite extracts. Furthermore, Pfcyc3 activates PfPK5 *in vitro*.

3.5. Novel FIKK kinases

Of all PfPKs identified, the presence of a novel family of 20 PKs is particularly noteworthy [42,90]. This family of kinases is termed FIKK based on a conserved amino acid sequence motif present [42]. All family members contain a non-conserved N-terminal domain and a conserved kinase domain in the C-terminus. The FIKK kinases contain all residues that are important for catalytic activity except the Glycine triad in subdomain I. The N-terminal domain is not conserved among paralogs and this region contains a stretch of hydrophobic residues corresponding to a predicted trans-membrane or signal sequence [90].A recently described host-targeting (HT) signal motif RxSRILAExxx [91] is present in six FIKK paralogs, whereas the *Plasmodium* export element (Pexel) RxLx(D, E, Q) [92] can be detected in all FIKK paralogs downstream of the signal sequence. Because HT/PEXEL motifs have been shown to mediate export of proteins beyond the parasitophorous vacuole into the erythrocyte cytoplasm [91,92], it is expected that FIKK kinases are trafficked to erythrocytes. Indeed, GFP-fusion protein of one of the FIKK kinases, FIKK12, was shown to be exported to the erythrocytes and associates with Maurer's clefts [93]. Although protein kinase activity of FIKK12 was detected in immunoprecipitates [93], recombinant FIKK 11 and FIKK 10.1 did not show any protein kinase activity using a peptide phosphorylation motif array [Turk and Chakrabarti, unpublished]. FIKK kinases may have a role in parasite-induced signaling events, given that members of this family are exported into the erythrocytes, associate with Maurer's clefts, and one of the paralogs, R45, is trafficked to the host cell membrane [94].

4. Concluding remarks

The knowledge of signaling transduction pathways in *Plasmodium* is fundamental to aid the design of new strategies against malaria. The finding that *Plasmodium* possesses serpentine receptors [38] opens new possibilities to dissect the upstream mechanisms through which *Plasmodium* senses the environment. On the other hand, the use of second messengers by parasites such as cAMP and calcium has long been suggested in the literature and finding their target could bring invaluable information regarding *Plasmodium* cell biology. Together with downstream mechanisms for signaling in *Plasmodium* they will provide a more complete picture of how *Plasmodium* signaling handling machinery is put in action.

Precise delineation of *Plasmodium* protein kinase functions as key regulators of cellular events will be a major challenge of the post-genome project era. It is apparent from earlier discussions

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that it will be difficult to ascertain physiological roles of Pf kinases simply based on homology because many of these proteins are expected to possess parasite-specific function as a means of regulating complex life cycle events. Therefore, characterization of physiological function of *Plasmodium* kinases will not be a mere repetition of what is already known in model organisms but will provide novel parasite specific information and fill a major gap in our understanding of the malaria parasite life cycle. Studies on the malarial protein kinases, their regulators and substrates will also provide new avenues of drug design targeting intraerythrocytic stages. Targeting protein kinase substrates rather than typical ATP-binding pocket will allow us to inhibit specific physiological events. Although targeting protein–protein interactions can be challenging because of complexity and diversity of binding surfaces, there have been recent progresses towards developing such therapeutic intervention approaches [95]. One such method is known as ‘fragment assembly’ that probes large chemical space as seen with interacting surfaces between proteins [96–98]. Alternatively, interfering peptidomimetics can also be developed. A long-term goal of this project is to use similar approaches can be utilized to identify molecular entity targeting malaria parasite kinases.

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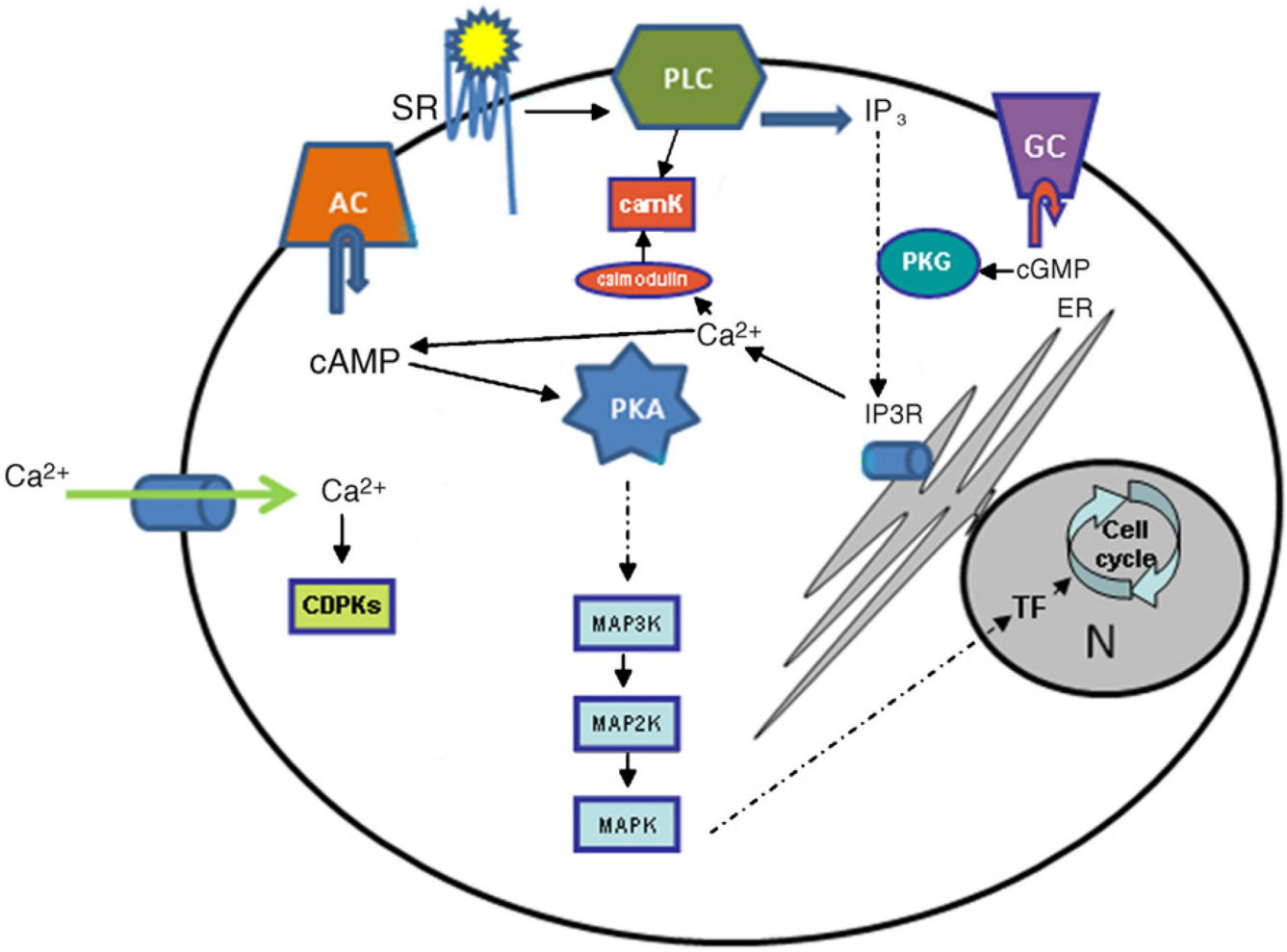

Fig. 1.

Schematic pathway of signaling in *Plasmodium*. AC adenylate cyclase, camK calcium/calmodulin kinase B, PDE phosphodiesterase, PLC phospholipase C, PKA protein kinase A, PKG protein kinase G PV parasitophorous vacuole, N nuclei, SR serpentine receptor. Tryptophan-derivatives are able to increase citoplasmic calcium through PLC. Calcium increase activates adenylate cyclase that convert AMP in cAMP once the concentration of such molecule is augmented in response to calcium increase by melatonin. cAMP is able to bind the regulatory subunit of PKA (cyclic AMP-dependent protein kinase) leading to an allosteric change in conformation which causes unleashing of the catalytic subunits becoming it activated and able to phosphorylate its targets. The molecular downstream effects of calcium and PKA in this pathway are proposed.