Signal Transduction in Malaria Parasites

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Over the past few years, several reports have been published about the characterization of Plasmodium *genes that are thought, on the basis of sequence homology with eukaryotic genes of known function, to be involved in the regulation of growth and differentiation of the parasite. Taken together with phenomenological observations on the regulation of developmental stages in the malaria life cycle, these data form the basis of an informative, albeit incomplete, picture of signal transduction in* Plasmodium. *Christian Doerig here reviews* Plasmodium *elements that are presumably part of major regulatory pathways conserved in eukaryotes, and addresses the problem of how to pursue such studies beyond the stage of gene identification.*

Members of the genus *Plasmodium*, like many other protozoan parasites, have a complex life cycle that is characterized by the succession of several specialized developmental forms, each of which is indispensable for the continuation of the cycle. Developmental stages differ from each other by morphological features, patterns of gene expression and cell division status. Some stages (asexual intraerythocytic forms, exflagellating male gametocyte, sporogonic oocyst and intrahepatocytic stages) are characterized by active cell division, whereas others (gametocytes, ookinete, sporozoite) undergo cell cycle arrest. The parasite must maintain a tight control over its cell cycle machinery, in order that the cell division status is coordinated with the state of differentiation at any developmental stage. How does the parasite know when to initiate development into the next stage? And how does it coordinate the diverse responses (eg. cell cycle progression or arrest, cell mobility, stage-specific gene expression) required for completion of the transition from one stage to the next, in accordance with environmental changes or internal stimuli?

Two main approaches have been employed to tackle these questions. The first involves the detection in the parasite of enzymatic activities known to mediate signal transduction in other eukaryotes. This strategy has been extended in recent years by a direct search for genes involved in signal transduction or in diverse adaptative responses. Candidate genes are isolated on the basis of homology with genes from other organisms in which their function is well characterized. The advent of polymerase chain reaction (PCR) techniques and the availability of expressed sequence tag (EST) banks[1] have greatly facilitated the search for specific genes. A shortcoming of this strategy is that it will miss any element specific to *Plasmodium*. The second approach consists of phenomenological studies aimed at determining the nature of signal transduction pathways involved in a given developmental process, such as erythrocyte invasion, gametocytogenesis or gametogenesis. The scope of this review is to present the picture of *Plasmodium* signalling elements that has emerged from a combination of these two approaches. Although the data gathered so far barely scratch the surface of the subject, they are nevertheless sufficient to suggest that many of the broad types of signalling systems found in other eukaryotes also operate in *Plasmodium*.

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Signal transduction pathways likely to operate in malaria parasites

This survey is restricted to pathways that available data suggest are present in malaria parasites. Furthermore, for the sake of clarity we will consider each pathway separately, although in reality extensive cross-talk occurs between different signal transduction routes. The discussion below is summarized in Fig. 1.

cAMP-dependent pathway. Protein kinase A (PKA) exists in eukaryotic cells as an inactive complex of catalytic and regulatory subunits. Binding of cAMP dissociates the complex and thereby activates the kinase function of the catalytic subunit[2], which then phopshorylates a number of substrates including transcription factors, causing changes in the gene expression pattern. cAMP levels are themselves controlled by the activity of the membrane-bound adenylyl cyclase, which, in turn, is activated by surface receptors, usually via heterotrimeric GTP-binding proteins (G proteins)[3].

cAMP is a key signalling molecule of many developmental processes in lower eukaryotes: it is used as a signal for sexual differentiation in *Chlamydomonas reinhardtii*[4] and as an aggregation signal by *Dictyostelium discoideum*; in the latter case, exogenous cAMP stimulates adenylyl cyclase via a cAMP surface receptor, thereby increasing intracellular cAMP levels[5]. Likewise, cAMP or activators of adenylyl cyclase enhance metacyclogenesis in *Trypanosoma cruzi*[6].

Plasmodium falciparum extracts have been shown to contain both adenylyl cyclase[7] and PKA[8] activities. The parasite's adenylyl cyclase shows properties distinct from those of mammalian homologues: it uses almost exclusively Mn^{2+}ATP instead of Mg^{2+}ATP as a substrate, and its activity is unaffected by compounds inhibiting G-protein activity (although evidence for the presence in the parasite of the α subunit of the heterotrimeric G protein is available, see below). This raises the question of how the pathway is activated in the parasite. cAMP has been implicated in *P. falciparum* sexual differentiation: Kaushal *et al.*[9] reported that addition of cAMP to high density parasite cultures triggered gametocytogenesis; reproduction of these results by other workers has proven difficult[10], making this issue controversial (gametocytogenesis is reviewed in Ref. 11). Data from other experimental approaches, nevertheless, support the idea of a role for a cAMP-dependent pathway in sexual differentiation: treatment of parasite cultures

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with caffein[12] and 8-bromo-cAMP[13], two phosphodiesterase inhibitors (which maintain cAMP levels artificially elevated), stimulated gametocytogenesis, and PKA activity was shown to be lower in clones unable to differentiate than in gametocyte producers, despite similar cAMP levels in both types of parasites[14].

Putative homologues of adenylyl cyclase and PKA genes have recently been identified in *Plasmodium*: a *P. falciparum* gene encoding a molecule with all the structural characteristics of adenylyl cyclases is currently being investigated (D. Baker, pers. commun.), and a gene encoding a PKA catalytic subunit homologue has been found in *P. yoelii*[15]; the *P. falciparum* version of PKA has also been identified (C. Doerig, D. Parzy and G. Langsley, unpublished; D. Lin and C. Syin, pers. commun.). Lin and Syin also report preferential accumulation of PfPKA mRNA in asexual parasites as distinct from gametocytes or gametes.

cGMP-dependent pathway. Elements of this pathway appear to be implicated in gametogenesis. This process occurs in the mosquito midgut and is triggered by cold shock and presence of the gametoctye activation factor (GAF) produced by the insect; the role of pH changes in gametogenesis is debated (reviewed in Ref. 16). Both cGMP and guanylyl cyclase activators appear to stimulate exflagellation in *P. falciparum* and *P. berghei*, whereas adenylyl cyclase activators have no effect on this process[17]. Treatment of male gametocytes with inhibitors of cAMP/cGMP-dependent kinases prevents morphological development of male gametes, but allows DNA synthesis to occur as in the controls[18]. Despite the problems of interpretation linked to the use of inhibitors that may affect other elements in addition to the desired target, this suggests that several signalling pathways are required for exflagellation, including one involving cGMP as a second messenger, the latter appearing not to regulate DNA synthesis. A gene encoding a protein kinase G from *P. falciparum*, whose transcripts are detectable in asexual intraerythrocytic parasites and to a lesser extent in gametocytes and gametes, is currently being characterized (C. Syin, D. Lin and N. Goldman, pers. commun.).

MAP kinase pathways. Several MAPKs (mitogen-activated protein kinases), also called ERK (extracellularly-regulated kinase), pathways co-exist in eukaryotic cells, and mediate responses to a wide variety of signals. In *Saccharomyces cerevisiae*, for example, at least five such pathways are known that are responsible for the cell's response to signals such as mating pheromone/receptor binding, cell wall alterations and osmotic pressure changes (reviewed in Ref. 19 and references therein). The central element in MAPK pathways is a module consisting of the MAPK itself, a MAPKK or MEK (MAPK/ERK kinase) whose function is to activate the MAPK by dual phosphorylation of both the threonine (T) and tyrosine (Y) residues in a TXY motif conserved in MAPKs, and a MAPKKK or MEKK which activates the MEK. The MEKs have very stringent substrate specificities and are able to phosphorylate only the appropriate MAPKs, whereas the MAPKs have many substrates whose phosphorylation is required for the adaptative response they mediate. MAPK substrates include transcription factors, cell cycle control elements, cytoskeleton components and downstream protein kinases. MAPK modules can be activated in a variety of ways, including G-protein-coupled receptors that can transmit the signal via the ras GTPase, receptor tyrosine kinases, and protein kinase C[20].

A MAPK homologue from *P. falciparum* has been identified independently in three laboratories[21–23]. Pfmap-1 (also called PfMAP or PfMRP) mRNA is present in asexual parasites[21], but levels appear to be elevated in gametocytes and gametes, suggesting a role in sexual development[22]. Three tyrosine-phosphorylated (and hence presumably activated) forms of Pfmap-1 are detectable in asexual parasites, indicating that the pathway to which it belongs is active at this stage in at least a subset of the cells[23]. This is consistent with preliminary experiments with a monoclonal antibody against the phosphorylated activation (TXY) site of MAPKs, which suggest that extracts from asexual parasites contain at least three forms of active MAPKs [whether these are the isoforms of Pfmap-1 cited above or the products of additional MAPK gene(s) is as yet unclear (C.D. Doerig and G. Langsley, unpublished)]. We are currently characterizing Pfmap-2, a *P. falciparum* gene encoding a kinase that is closely related to MAPKs, although the supposed activation site consists in this enzyme of a threonine-serine-histidine motif (TSH) (C.D. Doerig and D. Parzy, unpublished).

An attractive hypothesis is that Pfmap-1 (or another MAPK) is involved in the developmental switch leading to gametocytogenesis, as there is evidence that the decision to differentiate is made during the round of asexual division that precedes invasion by the gametocyte-to-be[24]. Events leading to Pfmap-1 phosphorylation are not known. Important activators of MAPK pathways in other systems are receptor-coupled heterotrimeric G proteins, which in most instances act through the ras GTPase[20]. Evidence for the presence of both an α-subunit of the G protein and for a ras-like polypeptide in asexual blood-stage parasites has been obtained from western blot experiments using antibodies against conserved regions of these molecules[25]. The ras-like protein is expressed throughout the asexual cycle, whereas the putative Gα subunit is detectable only in mature schizonts. Whether these elements function in the upstream part of signal transduction pathways remains to be confirmed. The tools are now available to determine if *Plasmodium* MAPK pathway activation is affected by treatment with compounds that interfere with G-protein function.

Phosphatidylinositol cycle. Many cellular responses to the occupancy of surface receptors include the hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP_2) by phospholipase C, with the subsequent generation of the second messengers inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG). IP_3 transmits the signal by increasing intracellular Ca^{2+} concentration through a release of Ca^{2+} stored in the endoplasmic reticulum, and DAG by activating protein kinase C. Phosphatidylinositol (PI) can be resynthesized from DAG by the PI synthase enzyme pathway. PIP_2 and related molecules also play important roles in the regulation of the cytoskeleton, as they are implicated in actin polymerization and depolymerization (reviewed in Ref. 26).

Exflagellation of *P. falciparum* gametes is accompanied by the sequential appearance of PI degradation

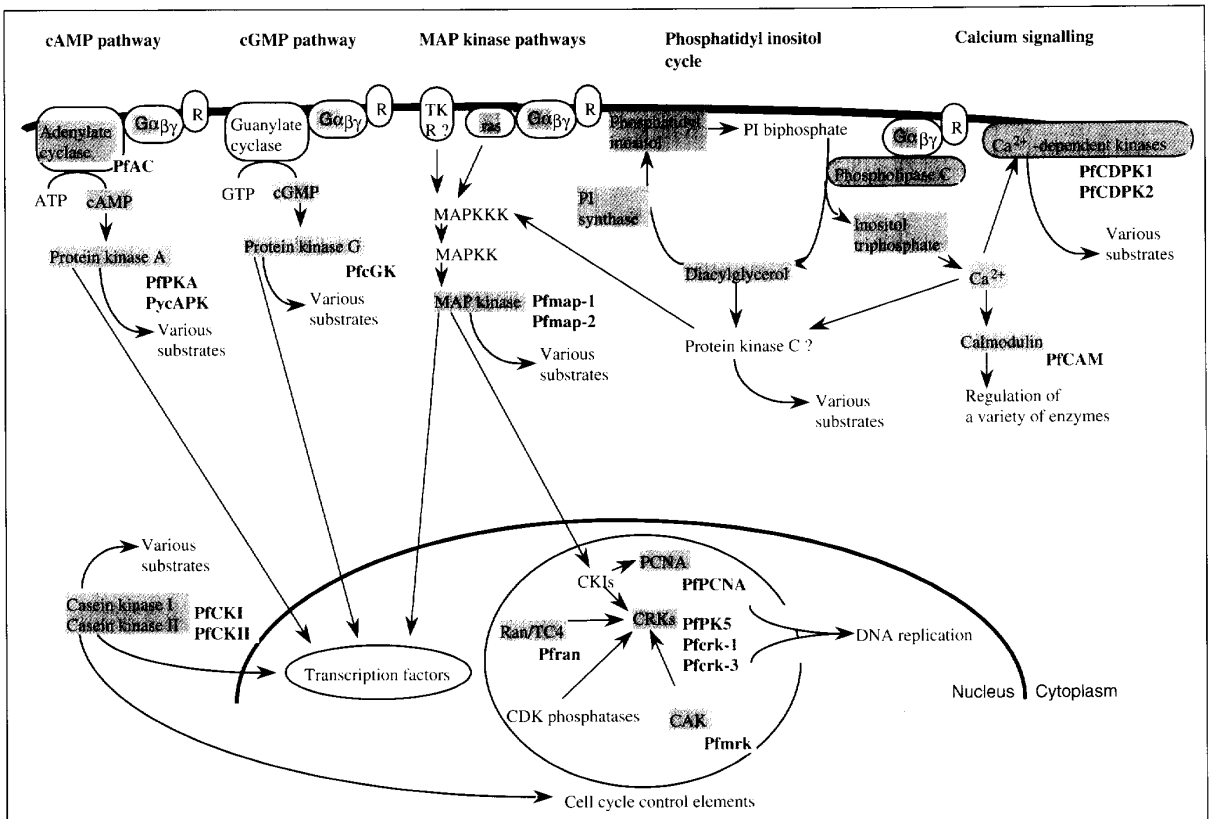

Fig. 1. Signal transduction pathways likely to operate in malaria parasites. Pathways are included in the figure only if: (1) a putative *Plasmodium* homologue has been identified for at least one gene product in the pathway; or (2) if they include second messengers that are thought to be involved in *Plasmodium* development. Such elements known to be involved in *Plasmodium* signalling are highlighted in red, and cloned *Plasmodium* homologues are indicated to their right in blue. The indicated position of *Plasmodium* gene products within the pathways is based solely on sequence comparisons with homologues from other organisms, as at the moment empirical evidence for their functioning in the proposed pathways is nonexistent. For the sake of clarity, all pathways are presented in an oversimplified way and without taking cross-talk into consideration; it should be borne in mind that the reality is far more complex. The term 'various substrates' includes enzymes that may themselves transmit a signal further downstream. Several kinases possibly acting in such a downstream position have been identified in *P. falciparum*, including the gametocyte-specific PfKIN[68], which shows homology to a group of stress-responding kinases; and casein kinases I and II, which in other systems play a role in the regulation of transcription factors, DNA metabolism and other cellular functions, and whose genes have been identified in *P. falciparum* (CKI: S. Barik and D. Chakrabarti, pers. commun.; CKII: C. Syin, pers. commun.). R, receptor; TK, tyrosine kinase.

products including IP_3 and DAG, indicating that the PI cycle is involved in this process and providing circumstantial evidence for phospholipase C activity (products of phospholipase A activity also appear during gametocyte activation)[27]. This is consistent with the finding that phospholipase C inhibitors interfere with exflagellation[28]. The presence of a complete PI cycle in *Plasmodium* is suggested from evidence for PI synthase activity in asexual parasites and the *de novo* appearance of PIP and PIP_2 in infected (but not in uninfected) erythrocytes[29].

Erythrocyte invasion also involves phospholipase activity: a protease that is bound to a membrane in the apical complex of the merozoite by a glycosylphosphatidylinositol anchor is solubilized (and hence activated) through the action of a GPI-phospholipase C in both *P. falciparum* and *P. chabaudi*[30]. The active, solubilized protease is required for merozoite entry into the red blood cell[31]. As this reaction releases DAG (a known activator of protein kinase C), it would be of interest to determine whether this event is functionally linked to the staurosporine-sensitive step that is essential for completion of the invasion process[32] (staurosporine is a protein kinase inhibitor with a rather broad specificity).

Calcium signalling. The importance of calcium in signal transduction is well established, and its indirect effects on the cell are widespread including cell cycle arrest, regulation of meiosis and regulation of transcription factors. A second source of Ca^{2+} apart from the endoplasmic reticulum is the extracellular medium, calcium entry into the cell being controlled by specific transmembrane channels. The calcium-binding protein, calmodulin, is an important mediator of calcium signalling and is required for the regulation of a wide variety of Ca^{2+}-dependent enzymes (reviewed in Ref. 33).

The correlation between PIP_2 hydrolysis and exflagellation discussed above suggests that calcium is a regulator of gametocyte activation. Indeed, calcium antagonists (compounds that inhibit Ca^{2+} release from the endoplasmic reticulum and inhibit calmodulin) interfere severely with gametogenesis in *P. falciparum* and *P. berghei*, preventing both normal morphological

development and DNA synthesis (in contrast to cGMP inhibitors, which impede development but allow DNA synthesis to occur normally)[18]. Extracellular calcium is not required for exflagellation, as shown by the use of chelating agents or Ca^{2+}-channel inhibitors[17]. Although these experiments were performed using an arguably unnatural trigger for exflagellation (incubation in an alkaline medium)[16], it is likely that Ca^{2+} also regulates gametocyte activation in the mosquito. Intraerythrocytic asexual growth is also inhibited by calcium inhibitors and calmodulin antagonists[34], and asexual parasites have been shown to contain Ca^{2+}-dependent protein kinase activity[7].

A *P. falciparum* homologue of calmodulin was first detected by immununological methods in asexual parasites[35], and the corresponding gene has subsequently been isolated[36]. A sexual stage-specific surface antigen considered as a potential target of transmission-blocking immunity, Pfs40, was shown to consist of an integral membrane protein with five copies of the so-called 'EF-hand' motif, which is shared among calcium-binding proteins; Pfs40 is indeed able to bind $^{45}Ca^{2+}$ (Ref. 37). Two genes encoding Ca^{2+}-dependent protein kinases, PfCDPK1 (Ref. 34) and PfCDPK2 (B. Kappes, pers. commun.) have been isolated from *P. falciparum*. Bacterially expressed forms of both enzymes phosphorylate proteins in a Ca^{2+}-dependent fashion, and both contain a calmodulin-like domain with four EF-hand motifs linked to the kinase catalytic domain and able to bind $^{45}Ca^{2+}$. At least one of these kinases (PfCDPK1) appears to be associated with the cell surface of the merozoite and is thought to play a role in erythrocyte invasion, perhaps by modifying proteins of erythrocytic origin[38]. PfCDPK2 may be associated also with a membrane or organelle fraction, as it contains a membrane-anchoring motif (B. Kappes, pers. commun.). CDPKs have been found in several plant species and in ciliates, but seem to be absent from vertebrates[39]; furthermore, as noted by Rawlings and Kaslow[37], EF-hand-containing proteins are usually cytosolic, with the documented exceptions of Pfs40, P24 (a protein with a possible role in invasion by *Toxoplasma gondii*), many plant CDPKs and PfCDPK1. These observations illustrate the phylogenetic relation of the Apicomplexans to the vegetal kingdom[40].

In line with this idea, it is noteworthy that Read and Mikkelsen came to the conclusion from their pharmacological data that *P. falciparum* may not possess a protein kinase C (PKC) homologue[8]. PKC isozymes are important mediators of calcium signalling in a wide variety of higher eukaryotes, but their presence in plants has been debated[41] (in *S. cerevisae*, a PKC homologue is present but does not respond to Ca^{2+} or DAG *in vitro*, despite similar substrate specificitiy to that of mammalian PKCs)[42]. Might the CDPKs, which are present in plants and protozoans but apparently not in vertebrates[39], be functional homologues of PKC in these organisms?

Protein phosphatases. Dephosphorylation of regulatory proteins is an important way of modulating the output of signal transduction cascades involving protein kinases[43]. For example, the activity of MAPK pathways is negatively regulated by protein phosphatases[44], whereas at least some cyclin-dependent kinases (CDKs) (see below) are inactive when phosphorylated on certain residues, and require activation by a protein phosphatase in order to become functional[43]. Response effectors themselves such as transcription factors can be inactivated by phosphatases[43]. Pharmacological evidence for phosphatase activity in merozoites has been reported[32], and more recently two different serine/threonine protein phosphatase activities (PP2A-like and PP1-like) have been detected in *P. falciparum* along with the identification of a putative protein phosphatase PP2A gene in *P. falciparum* (S. Barik and D. Chakrabarti, pers. commun.). Phosphatase PP2A is known to downregulate MAPK activity in other systems[44]. The fact that cloned *P. falciparum* puta-tive homologues of both MAP kinases and PP2A are now available suggests some straightforward experiments.

An effector of signal transduction: the cell cycle machinery

Regulators of cell cycle progression are important targets in signalling pathways that mediate cell growth and differentiation, and constitute in themselves an intricate signalling system. In *Schizosaccharomyces pombe*, entry into mitosis (M phase) and DNA synthesis (S phase) is controlled by the $p34^{cdc2}$ CDK, whereas in higher eukaryotes cdc2 homologues (called CDK1) control only entry into M-phase, the other phases of the cell cycle being regulated by additional CDKs. CDKs are regulated not only by ligand binding (positively by cyclins and negatively by CDK inhibitors, see below), but also by their phosphorylation status: their activation requires phosphorylation by a CAK (CDK-activating kinase) on precise residues and dephosphorylation on others by a phosphatase (reviewed in Ref. 45 and references therein). As an example of how progression of the cell cycle can be controlled by extracellular signals, one can consider the yeast mating pheromone response: pheromone binding to its surface receptor activates an MAPK pathway, which results in the phosphorylation of a CDK inhibitor (a CKI). The latter then binds to the CDK/cyclin complex, causing cell cycle arrest[46]. A key CKI in mammalian cells is p21, found in association with CDK/cyclin complexes and with PCNA (proliferative cell nuclear antigen), which is a processivity factor for DNA polymerase δ and is required for loading the enzyme onto DNA. Association with a CKI/CDK/cyclin complex sequesters PCNA, thus inhibiting polymerases already engaged in DNA synthesis and presumably preventing new polymerases from being loaded onto the DNA, thereby contributing to cell cycle arrest during differentiation (reviewed in Ref. 45).

A putative cdc2 homologue, called PfPK5, has been characterized in *P. falciparum*[47]; although *cdc2*-mutant complementation by PfPK2 was unsuccessful (perhaps because of problems linked to heterologous expression), mutagenesis experiments suggest that this enzyme can be partially activated *in vitro* by the same phosphorylation event as yeast cdc2 (Ref. 48). The recent isolation of a putative *P. falciparum* CAK homologue[49] is consistent with the hypothesis that CDK regulation in malaria parasites is similar to that observed in other eukaryotes. Additional *P. falciparum* cdc2-related kinases have been identified. Pfcrk-1 (Ref. 50) shows highest homology to the PISTLRE family,

some members of which act as downregulators of cell division. As Pfcrk-1 mRNA appears to accumulate preferentially in non-dividing gametocytes, it has been hypothesized that this enzyme is involved in sexual differentiation. Pfcrk-3, a novel kinase with homology to CDKs and MAPKs, is awaiting complete characterization (C.D. Doerig and D. Chakrabarti, unpublished), and this will probably not be the last member of this family of enzymes to be identified in the parasite. Hence *P. falciparum* appears to use a rather large number of cdc2-related kinases, in line with other parasitic protozoans[51].

The genes encoding several polypeptides involved in DNA replication, including PCNA, have been identified in *P. falciparum* (reviewed in Ref. 52). Initiation of DNA replication in eukaryotes is controlled by replication licensing, which ensures that DNA replication occurs only once per cycle (reviewed in Ref. 53). Major players in this control are the MCM (minichromosome maintenance) proteins and cdc18, of which *P. falciparum* homologues are currently being investigated (D. Chakrabarti, pers. commun.). Likewise, a *P. falciparum* homologue of the Ran/TC4 protein has been characterized[54,55]. Ran/TC4 is a nuclear GTPase related to ras and responsible (among several other functions) for transmitting a signal that prevents the onset of mitosis before DNA replication is completed, possibly by indirectly inhibiting cdc2 through the modification of its phosphorylation status[56]. In a recent discussion of the cell cycle in malaria parasites[57], it was suggested that division proceeds independently in each nucleus of the schizont, each nucleus importing the CDK/cyclin complex it requires at any given time (the *Plasmodium* nuclear membrane presents the peculiarity of remaining intact during nuclear division)[58]. Considering that one of the functions of Ran/TC4 in other systems is to mediate protein import into the nucleus[59], it would be of interest to determine whether Pfran (whose RNA accumulates to peak levels in late trophozoites[54] or schizonts[55]) is required for this process.

It is clear from the above discussion that there is a growing list of available molecular tools to investigate cell cycle control in *Plasmodium*, and one can expect significant progress to be gained in this field over the next few years.

Towards an integrated picture of signalling in malaria parasites

Missing links. Fundamental pieces of our puzzle are missing. Firstly, the precise signals which trigger developmental responses are for the most part unknown. Available data on parasite signal transduction concern essentially gametocytogenesis (reviewed in Ref. 11), gametocyte activation (reviewed in Ref. 16) and erythrocyte invasion, but there are many more points and processes in the life cycle that must involve regulated cell responses. Secondly, information regarding the structure of transduction pathways used by the parasite is, at best, fragmentary. Identification and functional analysis (see below) of the following elements would significantly increase our understanding.

(1) Surface receptors: molecules that sense the extracellular signals and initiate signal transduction cascades have not been functionally identified. However, it is likely that genes encoding such receptors have already been cloned: there is no reason *a priori* to think that some of the numerous surface antigens characterized at all developmental stages in the context of immunological studies might not function as signal receptors. It is noteworthy that no *Plasmodium* tyrosine kinase has yet been identified; tyrosine kinase activity is displayed by several surface receptors in other systems, where they act as initiators of important signalling pathways. Completion of the budding yeast genome project has shown that no typical tyrosine kinases are to be found in this organism, although tyrosine phosphorylation occurs in yeast, essentially as the result of the activity of dual-specificity kinases[60]; it has been speculated that true tyrosine kinases arose when multicellular organisms evolved and intercellular communication needed the development of a novel signalling system[60]. Given the considerably more complex life cycle of malaria parasites compared with that of yeast, it will be of interest to ascertain the presence or absence of enzymes of this class in *Plasmodium*.

(2) Stage-specific transcription factors: these form an essential group of response effectors, and represent one of the targets of many signalling pathways. Little information is available on the machinery regulating stage-specific transcription in malaria parasites, although nuclear run-on experiments indicate that at least some stage-specific gene expression changes are regulated at the level of RNA synthesis[61]. To our knowledge, the only *Plasmodium* transcription factors identified to date are homologues of the general transcription factors TBP (TATA-box binding protein)[62], and SNF2, a protein involved in disrupting chromatin structure and thereby allowing transcription initiation factors to bind to the DNA (D. Ji and D. Arnot, pers. commun.). A *P. falciparum* protein homologous to the Tat-binding protein family, some members of which mediate transactivation of transcription by other factors, has been identified and shown to be located in the nucleus[63]. The precise function of these elements in the regulation of transcription in unknown, and whether or not these proteins are direct targets of signalling pathways remains to be tested experimentally.

(3) CKIs and cyclins: detection of such molecules would be very informative in the context of the interface between signal transduction and cell cycle control.

The search for some of these missing gene products will be facilitated by the availabilty of sequences encoding proteins thought to interact with them, as one can exploit protein–protein interactions to identify novel genes involved in a given pathway[64].

Functional analysis. Although informative to some extent by itself, gene identification needs to be complemented by an analysis of the function each gene fulfils in the parasite. The availability of cloned genes greatly facilitates experiments aimed at understanding the role of given proteins, for example through the use of heterologously expressed gene products or monospecific antibodies. With such tools it becomes relatively straightforward to monitor the activation of specific gene products during development, for example by determining their phosphorylation state. In a similar way, pathways can be reconstructed by

studying the effect of putative upstream element inhibitors (such as compounds inhibiting G-protein-mediated transduction) on gene products suspected to act downstream in the same pathway.

In vivo, the function of cloned genes might be investigated by complementation of mutants from other organisms, or by modifying the parasite's genome itself. The recent demonstration of stable transformation in *P. falciparum*[65] suggests that a reverse genetics approach to address these questions is becoming available.

The ultimate objective: to prevent growth and/or development of the parasite

Most signalling pathways are essential to the cell and, therefore, represent attractive targets for chemotherapy. Some success has been achieved in reverting the transformed phenotype of tumor cells by way of chemical inhibition of signal transduction components[66]. Although both parasite and the host signalling systems share many common characteristics there are, nevertheless, differences which may potentially be exploited: primary structure differences between the parasite and host protein (as in PKA, MAPKs or Ran/TC4), or the presence in the parasite of CDPKs (a class of kinases that may be absent in the host cell), for example. Known inhibitors of kinases or other enzymes acting in signal transduction cannot be used directly as antimalarial drugs because of their effect on the vertebrate host. However, it has been shown that compounds inhibiting an essential function in both parasite and host can be chemically modified into related molecules that show differential activity on the parasite versus the host function. An example is a potential antimalarial topoisomerase II drug based on 9-anilinoacridine (a vertebrate topo II inhibitor): a slight modification of this molecule increased its parasite-specific effect by a factor of two to three orders of magnitude *in vitro*[67]. This leads one to consider a similar approach using signal transduction elements as targets. Whether or not this strategy will be successful, we do not know, but it certainly merits investigation.

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Mapping Malaria Risk in Africa:
What can Satellite Data Contribute?

M.C. Thomson, S.J. Connor, P. Milligan and S.P. Flasse

Recent developments in the access of remotely sensed vegetation and weather data, and their analysis along with other data sources within a geographical information system (GIS), have opened up new possibilities for African health services and research institutes in malaria stratification, monitoring and early warning. Madeleine Thomson, Stephen Connor, Paul Milligan and Stephane Flasse review the current situation and outline the way ahead.

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The recent successes in reducing child mortality by using insecticide-treated bednets (ITBNs)[1] have resulted in the adoption of this technology as a major focus for malaria control efforts in Africa[2]. In an earlier article in *Parasitology Today*, Snow and Marsh[3] suggest that the long-term effects of such interventions on cohort mortality in areas of high *Plasmodium falciparum* transmission are unknown, and that a