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The ins and outs of phosphosignalling in *Plasmodium*: Parasite regulation and host cell manipulation

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ABSTRACT

Signal transduction and kinomics have been rapidly expanding areas of investigation within the malaria research field. Here, we provide an overview of phosphosignalling pathways that operate in all stages of the *Plasmodium* life cycle. We review signalling pathways in the parasite itself, in the cells it invades, and in other cells of the vertebrate host with which it interacts. We also discuss the potential of these pathways as novel targets for antimalarial intervention.

1. Introduction

All eukaryotic cells rely on intracellular signalling to (i) coordinate the hugely complex processes of cellular life, such as cell division and differentiation and, (ii) respond to extracellular signals by mobilizing the appropriate response. Once a signal is sensed by a receptor, it is transmitted to effector machinery through conserved signalling mechanisms, that include (i) production/release of soluble second messengers such as cyclic nucleotides or calcium, (ii) recruitment of downstream pathway elements to specific sites, and (iii) protein modifications. Of the latter, reversible protein phosphorylation by protein kinases and protein phosphatases is undoubtedly the most prominent. Signal transduction pathways whose core components are protein kinase cascades represent a major mechanism of cell signalling in eukaryotes. Research over the last two decades clearly indicates that malaria parasites are no exception.

In line with the importance of protein phosphorylation in eukaryotic life, genes encoding protein kinases make up about 2% of the mammalian and yeast genomes [1,2]; this is similar in malaria parasites, whose kinome comprises 85–99 genes encoding protein kinases [3–6] in a genome totalling approximately 5,300 genes. The number of phosphatase catalytic domain sequences (the phosphatome) is somewhat smaller, with approximately 30 members [7,8]. Systematic reverse genetics studies in *P. falciparum* [9] and *P. berghei* [8,10] have shown that a large proportion of these enzymes are essential at various stages of the parasite's life cycle, in both the vertebrate host and the mosquito vector; furthermore, a recent high-throughput reverse genetics study of the *P. berghei* kinome, based on pools of barcoded mutants, revealed that several protein kinases function redundantly in asexual blood stages [11]. Taken together with data from a growing number of phosphoproteomic studies in blood [12–15] or mosquito [16] stages, and from detailed studies targeted at specific phenomena or kinases/phosphatases (see below), these provide an emerging picture of how phosphosignalling regulates most aspects of the parasite's life cycle (reviewed in [17]; Figs. 1 and 2; see Tables 1 and 2 for a list of all signalling molecules discussed in this review). Recent investigations have provided strong evidence that phosphosignalling not only plays a crucial role during infection in the parasite itself, but also in its host

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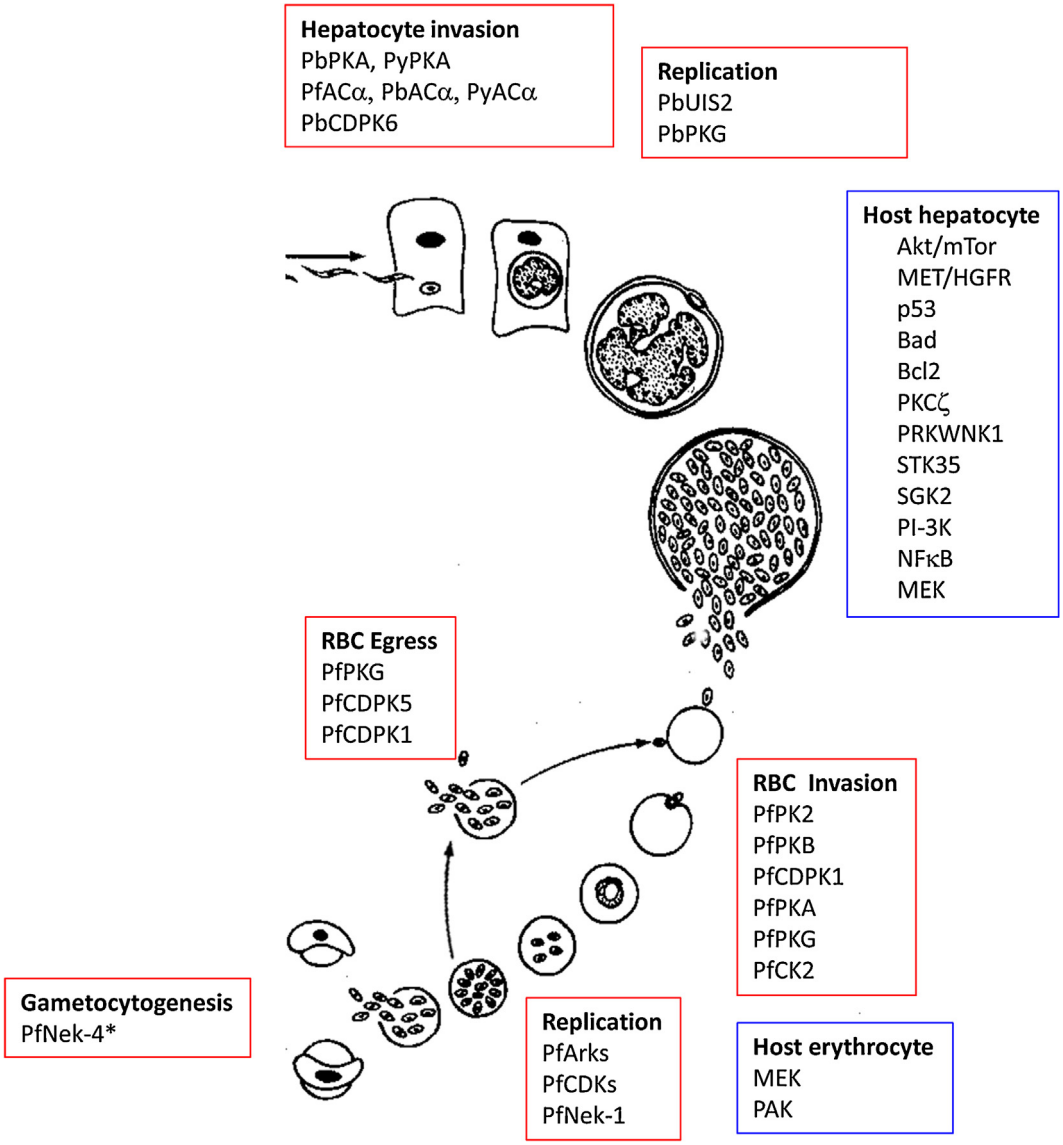

Fig. 1. Parasite (red boxes) and host cell (blue boxes) kinases essential for parasite development throughout its life in the human host. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
*Pfnek-4 is a marker of committed schizonts and developing gametocytes but is not essential for gametocytogenesis; Pbnek-4 is essential for meiosis in the mosquito vector.

cells [18–21] and in bystander cells such as endothelial cells of the vertebrate host and the mosquito vector (see below). Salient features of this fundamental aspect of the biology of malaria parasites are discussed in the ensuing sections.

2. Inside the *Anopheles* mosquito

2.1. Signalling in the parasite

Signalling pathways involve a dynamic interplay between protein kinases and protein phosphatases and play significant roles in sexual parasite development in both the human and mosquito host. In the human host, sexual differentiation is initiated when a subpopulation of merozoites divert from the asexual blood cycle and instead enter the sexual cycle to form gametocytes (see below).

Once in the mosquito, the combination of a drop in temperature by 5 °C (or more, depending on the species of *Plasmodium*), a rise in pH from 7.4 to 7.8–8.0, and the presence of xanthurenic acid, triggers gametocyte activation and differentiation into gametes [22–25]. The first visible step of gametocyte activation is the rounding up of male and female gametocytes, which is mediated by cGMP and the cGMP-dependent effector kinase, protein kinase G (PfPKG) [26–28]. PKG controls the calcium spike that is the earliest biochemical sign of activation in *P. berghei* gametocytes, and signals to downstream calcium-dependent kinases that control various effector mechanisms (see below) [16]. The male gametocyte, which has a DNA content of slightly more than 1C [29], undergoes three rounds of DNA replication during gametogenesis, to reach an 8C DNA content. Following axoneme assembly, each activated male gametocyte produces eight motile male gametes in a process termed exflagellation [29–32]. Several kinases and phosphatases have been identified as essential to successfully complete exflagellation. Calcium-dependent protein kinase 4 (PbCDPK4) is necessary for DNA replication and axoneme assembly [33] and mitogen-activated protein kinase-2 (PbMAP2) is critical for either axoneme maturation or cytokinesis that results in release of the 8 male gametes [34]. Interestingly, two NIMA-related protein kinases, PfNEK-1 and PfNEK-3, phosphorylate MAP2 *in vitro*, suggesting they may also play a role in this process [35–38]. The serine/arginine-rich protein kinase (PbSRPK/PfCLK4) and metallo-