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Review

Nima- and Aurora-related kinases of malaria parasites ☆

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ABSTRACT

Completion of the life cycle of malaria parasite requires a succession of developmental stages which vary greatly with respect to proliferation status, implying a tightly regulated control of the parasite's cell cycle, which remains to be understood at the molecular level. Progression of the eukaryotic cell cycle is controlled by members of mitotic kinase of the families CDK (cyclin-dependent kinases), Aurora, Polo and NIMA. *Plasmodium* parasites possess cyclin-dependent protein kinases and cyclins, which strongly suggests that some of the principles underlying cell cycle control in higher eukaryotes also operate in this organism. However, atypical features of *Plasmodium* cell cycle organization and important divergences in the composition of the cell cycle machinery suggest the existence of regulatory mechanisms that are at variance with those of higher eukaryotes. This review focuses on several recently described *Plasmodium* protein kinases related to the NIMA and Aurora kinase families and discusses their functional involvement in parasite's biology. Given their demonstrated essential roles in the erythrocytic asexual cycle and/or sexual stages, these enzymes represent novel potential drug targets for antimalarial intervention aiming at inhibiting parasite replication and/or blocking transmission of the disease. This article is part of a Special Issue entitled: Inhibitors of Protein Kinases (2012).

1. Introduction

Malaria is caused by infection with protozoan parasites of the genus *Plasmodium*, *P. falciparum* being the most virulent species in humans and responsible for the vast majority of lethal cases. 40% of the world's population is at risk, and 500 million of clinical cases as well as 800,000 deaths are reported yearly [1]. Recent drops in mortality followed the introduction of combination therapies involving artemisinin derivatives, preventive drug treatment, and mosquito control strategies [2]. However, the rapid emergence and spread of resistance against the available anti-malarial armamentarium (including artemisinin-derived compounds) urgently call for the development of new chemotherapeutic agents [3,4]. Research on malaria parasite biology, and in particular on asexual blood stages (the multiplicative stage of the life cycle associated with clinical manifestations) may lead to the development of new therapeutic strategies. In view of the recent successes in targeting protein kinases (PKs) in the context of cancer and other major diseases, the *P. falciparum* kinome has been proposed as an attractive potential target for novel antimalarials [5].

1.1. The Plasmodium cell cycle

The life cycle of malaria parasites alternates developmental stages characterized by intense cell division (e.g., the asexual multiplication in hepatocytes and erythrocytes in the human host, and the generation of sporozoites in the oocyst during development in the mosquito vector), and phases where the cell cycle is arrested and differentiation occurs (e.g., during gametocytogenesis), implying the existence of an efficient cell cycle control machinery. How *Plasmodium* regulates cell growth and division remains, however, largely unknown. The cell cycle of *Plasmodium* during intra-erythrocytic proliferation (the phase of the life cycle that is most amenable to experimental investigations) is unusual in many respects [6]. After the growth phase that follows invasion of the erythrocyte by the merozoite, and which brings the parasite from the early "ring" form to the trophozoite stage where DNA synthesis is initiated, successive nuclear divisions occur in the absence of cell division, leading to a multinucleated schizont. Another peculiarity is that nuclei in a given schizont divide asynchronously, and nuclei maintain their individuality, since the nuclear envelope is maintained throughout the nuclear division process (reviewed in [7,8]). *Plasmodium* has no typical centrioles/centrosomes. Instead, the mitotic spindles are emitted from spindle pole bodies (SPBs) that are associated with the internal side of the nuclear envelope. A clear correspondence between the G1, S, G2 and M phases has not been established, although it is agreed that rings and early trophozoites are in G1, and S is initiated in mature trophozoites. The organization of the cell cycle phases in dividing nuclei during schizont maturation is as yet not

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understood [8]. Mitosis in eukaryotic cells involves the concerted action of several conserved serine/threonine protein kinases known as mitotic kinases [9], which include cyclin-dependent kinases, Polo-like kinases, Nima-related kinases and Aurora kinases.

1.2. The Plasmodium kinome

Using a variety of bio-informatic tools, 85–99 genes encoding protein kinase-related enzymes in the *P. falciparum* genome were identified, which revealed a picture of profound divergence between *Plasmodium* and mammalian PKs [10,11]. Furthermore systematic reverse genetics of the entire kinome has been implemented in both the rodent malaria parasite *Plasmodium berghei* [12] and *P. falciparum* [13], leading to the establishment of a list of enzymes essential for asexual proliferation or for specific stages in sexual development. In the present review we focus on the Nima-related and the Aurora kinase families, both of which are represented with several enzymes in the *P. falciparum* kinome.

2. The eukaryotic NIMA and Aurora kinase families

2.1. NIMA kinases

The NIMA-related protein kinases (Neks) constitute a family of protein kinases initially identified as playing a variety of roles in cell cycle regulation (for reviews, see [14,15]). These proteins are widely represented in eukaryotes, and the first member of the NIMA (*N*ever *I*n *M*itosis A) family was discovered in a screen for cell cycle mutants in the fungus *Aspergillus nidulans* [16]. The single *Aspergillus* NIMA kinase is essential for mitotic entry and localizes to the spindle pole body (SPB), the major microtubule organizing center (MTOC) in fungi and apicomplexan parasites, and functional equivalent of the centrosome in higher eukaryotes. NIMA activity is required to promote localization of the Cdc2/cyclin B complex to the SPB and is thought to participate in its nuclear uptake through the nuclear pore via the modification of nuclear pore components [17,18]. NIMA can be phosphorylated by Cdc2/cyclin B, suggesting a positive feedback activation loop. The single NIMA-related kinase (Nek) of fission and budding yeasts, fin1 and Kin3, respectively, is not essential for mitotic entry in these organisms. However, it is an important cell cycle regulator with roles in the timing of mitotic entry, chromosome condensation and mitotic exit [14]. Fungal Neks also participate in nuclear envelope fission [19]. In mammals, there are eleven paralogous *Nek* genes, several of which, including those encoding the Nek2, Nek6, Nek7 and Nek9 kinases, have been reported to play roles in cell cycle regulation and/or to localize to centrosomes [14]. The most closely related in sequence and function to the fungal Neks is Nek2 [15,20]. Nek2 is a component of the centrosome at the time of mitotic entry and appears to initiate the separation of centrosomes at the G2/M transition and to enable bipolar spindle formation. The Nek2 kinase phosphorylates at least two proteins (C-Nap1 and rootletin) involved, in G2 cells, in the cohesion of duplicated centrosomes [21,22], thereby triggering their dissociation, and might prime the centrosomal protein Ninein-like protein (Nlp) for phosphorylation by the mitotic Polo-like kinase 1 (Plk1) [23]. Negative regulation of Nek2 autophosphorylation and activation is mediated through protein phosphatase 1 (PP1) [24]. PP1 enzymatic activity is conversely downregulated by Nek2, making a mutually antagonistic complex. Rapid increase of Nek2 activation is triggered upon inhibition of PP1 by the inhibitor-2 protein at the onset of mitosis [25]. Apart from centrosomal functions, Nek2 has other roles in mitotic progression, such as chromatin condensation, at least in meiotic spermatocytes. In view of its interaction with the core kinetochore protein Hec1, a protein required for recruitment of spindle checkpoint proteins to the kinetochore, Nek2 might participate to mitotic spindle checkpoints [26]. In the unicellular biflagellate *Chlamydomonas*, members of the Nek family were shown to regulate flagellar length and to promote disassembly of cilia [27]. Indications of the ciliary functions of some Neks in mammals come from mutations of the Nek1 and Nek8 kinases underlying ciliopathy observed in mouse models of polycystic kidney disease (PKD) [28,29]. Since Nek1 localizes to centrosome in interphase and mitosis, and since Nek8 is most closely related to Nek9, which functions in mitosis, there are indications in support of both cell cycle and ciliary functions for this enzyme. In view of the proliferation of kidney cells that lead to renal cyst formation in PKD, O'Regan and colleagues raised the interesting hypothesis that Nek kinase signaling might determine cell fate with respect to differentiation and mitotic proliferation [15]. Much larger numbers of Neks have been found in the genome of the ciliate *Tetrahymena* (40 genes) [30], as well as in the genomes of the excavates *Trypanosoma*, *Leishmania* [31] and *Giardia*; the genome of the WB strain of *Giardia lamblia* contains an astonishing 198 Neks, making up to 71% of its kinome [32]. It is interesting to note that all these unicellular organisms rely on motility based on complex flagellar machinery. Comparative examination of the genomes of several organisms reveals that Neks are more abundant in organisms with ciliated cells, and in which ciliary assembly and disassembly are coordinated to the cell cycle [33,34]. It has thus been proposed that expansion of the Nek family is related to evolution of a complex system coordinating the cell cycle with the dynamics of cilia, basal bodies and centrioles. Basal body/centrosomal localization of several Neks studied in these organisms is similar to patterns seen in *Metazoa* and fungi.

2.2. Aurora kinases

Aurora kinases are serine/threonine kinases that also play pivotal roles in the control of cell division. They have been described in various organisms, and their functions are closely linked to the dynamics of the centrosome and bipolar microtubule spindle as well as to chromosome segregation and cytokinesis. The first Aurora kinase was discovered in *Drosophila* in 1995 during a phenotype screening for mitotic spindle defects [35]. The loss of function of the kinase led to failure of the centrosomes to separate and to form a bipolar spindle. Since then, a range of Aurora-related kinases have been described in various organisms, including IpL1 (*I*ncrease-in-*pl*oidy protein *1*) in *Saccharomyces cerevisiae*, Ark1 (*A*urora-related *k*inase) in *Schizosaccharomyces pombe*, two members in *Drosophila* and *Caenorhabditis elegans*, and three in mammals, Aurora A, Aurora B and Aurora C [9]. In metazoans, two distinct Aurora family members, Aurora A and Aurora B, are expressed in all cell types, where they regulate cell cycle progression from G2 to cytokinesis, and both are overexpressed in several cancer cell types. Despite their high degree of mutual sequence homology, Aurora A and Aurora B display distinct localizations and functions.

The localization of Aurora A, also called the polar Aurora, varies during cell cycle progression, being associated with duplicated centrosomes during late S/early G2 and moving to the spindle poles in early mitosis. Aurora A plays a major role in centriole duplication, centrosome separation and maturation, and mitotic spindle formation. Some of these mechanisms appear to result from Aurora A activity on the kinesin-related protein Eg5 (reviewed in [36]). In order to become fully functional after they duplicate and separate, centrosomes must recruit a number of different proteins in a process known as maturation. In the absence of Aurora A, recruitment of several components of the pericentriolar material (including γ-tubulin) to the centrosome is deficient, and the microtubule mass of spindles is decreased by about 60% [37,38]. Aurora A regulation is complex and involves the interplay between the small GTPase Ran and the centrosome-associated protein TPX2 (*T*argeting *P*rotein for *X*KLP*2*) [39]. During mitosis, Ran-GTP releases TPX2 from Importin-α and Importin-β, allowing TPX2 to bind Aurora A and target it to the spindle polar microtubules [40]. TPX2 also regulates the kinase activity of Aurora A, both protecting it from the inhibitory action of the protein phosphatase PP1 (the same phosphatase that antagonizes Nek activity, see above) and by stimulating Aurora A autophosphorylation at Thr^{295}, a residue in the activation loop whose phosphorylation is essential for kinase activity [39,41].