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Giardia lamblia aurora kinase: A regulator of mitosis in a binucleate parasite

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Abstract

Giardia lamblia is a major cause of diarrhoeal disease worldwide. Since it has no known toxin, the ability of trophozoites to colonise the human small intestine is required for its pathogenesis. Mitosis in this protozoan parasite is a unique challenge because its two equivalent nuclei and complex cytoskeleton must be duplicated and segregated accurately. Giardial mitosis is a complex and rapid event that is poorly understood at the cellular and molecular levels. Higher eukaryotes have one to three members of the highly conserved Ser/Thr aurora kinase (AK) family that regulate key aspects of mitosis and cytokinesis. *Giardia* has a single AK orthologue (gAK) with 61% similarity to human AK A. In addition to the conserved active site residues, activation loop and destruction-box motifs characteristic of AKs, gAK contains a unique insert near the active site region. We epitope-tagged gAK at its C-terminus and expressed it under its own promoter. During interphase, gAK localises exclusively to the nuclei, but is not phosphorylated as shown by lack of staining with an antibody specific to phosphorylated AK A (pAK). In contrast, during mitosis pAK localises to the basal bodies/centrosomes and co-localises with tubulin to the spindle. During specific stages of mitosis, giardial pAK also localised dynamically to cytoskeletal structures unique to *Giardia*: the paraflagellar dense rods of the anterior flagella and the median body, whose functions are unknown, as well as to the parent attachment disc. Two AK inhibitors significantly decreased giardial growth and increased the numbers of cells arrested in cytokinesis. These inhibitors appeared to increase microtubule nucleation and cell-ploidy. Our data show that gAK is phosphorylated in mitosis and suggest that it plays an important role in the *Giardia* cell cycle. The pleiotropic localisation of AK suggests that it may co-ordinate the reorganisation and segregation of tubulin-containing structures in mitosis. We believe this is the first report of a signalling protein regulating cell division in *Giardia*.

Keywords: *Giardia lamblia*; Aurora kinase; Mitosis; Cytokinesis; Cytoskeleton

1. Introduction

Cell division is necessary for protozoan pathogens to colonise and cause disease. *Giardia lamblia* causes diarrhoeal disease that affects approximately 10% of the world's population (Adam, 2001). Motile *Giardia* trophozoites colonise the upper small intestine. Each half-pear-shaped cell has two nuclei and a unique cytoskeleton that is essential for attachment and survival in the intestine. Adhesion is dynamic, as trophozoites must detach from sloughed off intestinal epithelial cells at the tip of the villi, swim against peristalsis and adhere to new epithelial cells. Four pairs of motile flagella, an attachment disc, a median body and the funis characterise the unique microtubule-based cytoskeleton. Each flagellum is anchored to a basal body (centrosome) and leaves the cell body at a different locus. Video sequence microscopy suggests that each pair of fla-

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gella has a different function (Nohynkova et al., 2006). The intracellular parts of the anterior, posterior–lateral and caudal pairs of flagella are accompanied by paraflagellar dense rods, whose protein compositions are sensitive to environmental signals (Abel et al., 2001; Gibson et al., 2006; Lauwaet et al., 2007). The median body is a non-membrane associated stack of microtubules in the middle of the cell body, whose function is not known (Piva and Benchimol, 2004). The funis is a poorly studied structure at the posterior end of trophozoites and is composed of microtubules that extend between the caudal flagella and posterior–lateral flagellar rods (Benchimol et al., 2004). Each trophozoite has a ventral disc containing microtubules and micro ribbons of β-giardin that mediates attachment to enterocytes (Holberton, 1973; Adam, 2001).

Giardia cell division is challenging; it requires the duplication and reorganisation of both nuclei and cytoskeletal structures, as well as their equivalent redistribution between the two daughters (Nohynkova et al., 2006; Sagolla et al., 2006; Tumova et al., 2007b). Cell division must be rapid because without a functional cytoskeleton, trophozoites would be carried downstream in the intestine. The mechanism of mitosis in *Giardia* has been controversial (Ghosh et al., 2001; Solari et al., 2003; Benchimol, 2004a,b; Sagolla et al., 2006) and mitotic spindles have only recently been documented (Nohynkova et al., 2000; Sagolla et al., 2006; Tumova et al., 2007b). Ultrastructural analyses show evidence of semi-open mitosis with two extranuclear spindles in laterally dividing trophozoites (Sagolla et al., 2006). Trophozoites do not lose or absorb their flagella during cell division, but flagella detach from the basal bodies in early prophase (Tumova et al., 2007b). Previously, alternative orientations of cell division involving nuclear cleavage by the adhesive disc was reported (Solari et al., 2003; Benchimol, 2004a). Moreover, another study revealed that trophozoites can divide in multiple orientations (Benchimol, 2004b). These data suggest that cell division in *Giardia* is complex and may utilise multiple mechanisms. The regulation of mitosis and cytokinesis in *Giardia* is poorly understood and to date, no signalling proteins have been implicated. The study of mitotic structures and associated molecules is a challenge as mitosis is rapid, the number of mitotic cells in non-synchronous cell populations is low, cells are motile and tend to detach during certain stages of mitosis and cytokinesis, and are therefore difficult to capture (Ghosh et al., 2001; Benchimol, 2004b; Tumova et al., 2007b).

Aurora kinases (AKs) are a family of conserved serine/threonine kinases that are essential regulators of cell division and are typically highly expressed at the gap 2 phase/mitosis (G2/M) stage of the cell cycle (Katayama et al., 2003; Fu et al., 2007). AKs direct a number of mitotic events such as centrosome duplication, chromosome condensation, spindle assembly and cleavage furrow formation in eukaryotes (Andrews et al., 2003; Carmena and Earnshaw, 2003). In metazoans, the AK family has three members: AK A, AK B and AK C. All have a short C-terminus containing a destruction-box (D-box), a conserved catalytic domain with an activation loop and an N-terminal region, whose length and sequence varies (Giet and Prigent, 1999; Carmena and Earnshaw, 2003). Despite their close sequence homology, AKs differ in their functions and localisations. The AK A family, or 'polar auroras', dynamically localise to the centrosomes and spindle microtubules and function in centrosome maturation and bipolar spindle formation (Carmena and Earnshaw, 2003). AK B, or 'equatorial auroras', localise to the spindle midzone and are essential for chromosome segregation and cytokinesis (Andrews et al., 2003; Carmena and Earnshaw, 2003; Ke et al., 2003). The AK C in mammals resemble AK B (Li et al., 2004). However, studies on the roles of AK in pathogenic protozoa are scarce. So far, AKs have been characterised in *Leishmania major* (Siman-Tov et al., 2001) and *Trypanosoma brucei* (Tu et al., 2006). Of the three *T. brucei* AKs, only procyclic TbAUK1 is involved in spindle formation, cytokinesis and organelle replication, while in the bloodstream form TbAUK1 is only involved in cytokinesis (Li and Wang, 2006; Tu et al., 2006).

Giardia's cytoskeleton is central to infection and its structural reorganisations must be tightly regulated during cell division. We identified a single giardial aurora kinase, called gAK, in the *G. lamblia* genome and hypothesised that it might be important in cell division. Here we demonstrate that gAK is phosphorylated only in mitosis and cytokinesis. Moreover, the phosphorylated form localises to both universal mitotic structures and to cytoskeletal elements unique to *Giardia* that have not previously been implicated in cell division. Based on its localisation throughout mitosis and cytokinesis, gAK may carry out characterised functions of traditional AK A and AK B families. We also validate gAK's role in cell cycle control by showing that two AK inhibitors reduced giardial growth and arrested cells in cytokinesis.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise stated.

2.2. Cell culture

Giardia lamblia isolate WB (ATCC #50803), clone C6, trophozoites were cultured in TYI-S33 medium (growth media; Diamond et al., 1978; Keister, 1983).

2.3. Identification of the giardial aurora kinase (gAK) gene

We searched the *Giardia* genome database (www.mbl.edu/Giardia, McArthur et al., 2000), for homologues of aurora kinases using human AK A and AK B sequences (gi:27923855 and gi:27805737, respectively) as the query with the *Giardia* BLAST function (gblast; Altsc-