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Research paper

Aurora B kinase is required for cell cycle progression in silkworm

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ABSTRACT

Aurora B kinase, a member of serine/threonine kinase family, is the catalytic subunit of the chromosomal passenger complex and is essential for chromosome alignment, chromosome segregation, and cytokinesis during mitosis. Here, we cloned the full-length cDNA sequence of silkworm *Aurora B* (*BmAurB*) gene and predicted that BmAurB protein contains a conserved S_TKc domain. Phylogenetic analysis between *BmAurB* and other Aurora kinases indicates that Aurora kinases may have evolved after separation between mammalian and insect, and prior to radiation of either mammalian or insects. RT-PCR examination revealed that the expression of the *BmAurB* gene was high in mitotic cycling gonads, moderate in mitotic cycling brain, and undetectable in endocycling silk gland during silkworm larval development. RNAi or inhibitor-mediated inhibition of the *BmAurB* gene in silkworm ovary-derived BmN4-SID1 cells disrupted cell cycle progression during mitosis and induced an accumulation of polyploid cells, cell cycle arrest at G2/M phase, chromosome misalignment, chromosome bridge, and bi-nucleation. Taken together, our results suggest that the *BmAurB* gene is required for cell cycle progression in silkworm.

1. Introduction

Aurora kinases are characterized as a novel subfamily of serine/threonine kinases and have been shown to play essential roles in the control of cell division in eukaryotes (Katayama et al., 2003; Fu et al., 2007; Goldenson and Crispino, 2015). What's more, the expression level and catalytic activity of Aurora kinases are highly elevated in many human cancers and can be used as a prognostic marker and therapeutic target (Fu et al., 2007; Kelly et al., 2011; Goldenson and Crispino, 2015). Aurora kinase was first discovered in the fruit fly, *Drosophila melanogaster* (Glover et al., 1995). To date, three members of Aurora kinases, namely Aurora A, B and C, have been identified (Nigg, 2001; Fu et al., 2007). All Aurora proteins share a high sequence similarity among eukaryotes (Goldenson and Crispino, 2015), and have a common catalytic domain of the serine/threonine kinase (S_TKc) that displays a protein kinase activity (Kimura et al., 1998). Moreover, the types of Aurora kinases vary in different organisms. For example, only one Aurora kinase exsits in the yeast, *Saccharomyces cerevisiae* (Chan and Botstein, 1993). The fruit fly and the nematode, *Caenorhabditis elegans* have two Aurora kinases, namely Aurora A and B (Fu et al., 2007). All three types of Aurora kinases can be found in mammals (Nigg, 2001).

Aurora A and B are required for the control of mitosis whereas Aurora C is mainly invoved in meiosis (Goldenson and Crispino, 2015). Interestingly, Aurora B is partnered with inner centromere protein (INCENP), survivin and Borealin to form a chromosomal passenger complex (CPC) that is critical for the kinetochore-microtubule attachment during cytokinesis (Terada, 2001; Vernos, 2004; Ruchaud et al., 2007; Vader et al., 2007). Functionally, Aurora B mainly acts at the transition from metaphase to the end of mitosis and regulates chromosomal condensation and segregation, spindle assembly checkpoint, and cytokinesis (Fu et al., 2007; Goldenson and Crispino, 2015). The expression downregulation or activity inhibition of Aurora B protein leads to failure in cytokinesis and formation of of polyploid cells in *Drosophila* and vertebrates (Giet and Glover, 2001; Mathieu et al., 2013; Zekri et al., 2015).

Aurora B functions in cell division through its phosphorylation on multiple substrates (Fu et al., 2007; Goldenson and Crispino, 2015). For example, Aurora B can phosphorylate histone H3 that is involved in chromatin condensation and separation during mitosis (Adams et al., 2001; Giet and Glover, 2001). Recent studyies in the fruit fly reported that Aurora B phosphorylates not only cyclin B to delay cytokinesis abscission in germ cells but also Lgl (Lethal giant larvae) protein to induce mitotic spindle orientation in epithelial cells (Mathieu et al., 2013; Bell et al., 2015). In addition, substrates for Aurora B also include centromere protein A (CENP-A), GTPase-activating protein MgcRacGAP, mitotic-centromere-associated kinesin (MCAK), histone 2A, survivin, nuclear distribution protein C (NudC), microtubule-binding protein Hec1, and Ataxin-10 (Zeitlin et al., 2001; Minoshima et al., 2003; Knowlton et al.,

Abbreviations: cDNA, complementary DNA; RT-PCR, reverse transcription polymerase chain reaction; ORF, open reading frame; UTR, untranslated region; RNAi, RNA interference; S_TKc, catalytic domain of the serine/threonine kinase; RACE, rapid amplification of cDNA end; CPC, chromosomal passenger complex; BLAST, basic local alignment search tool.

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2006; Delacour-Larose et al., 2007; Zhu et al., 2013; Tian et al., 2015; Weiderhold et al., 2016).

To date, Aurora B kinase has not been well characterized in the silkworm, *Bombyx mori*. The role of silkworm Aurora B kinase (referred to hereafter as BmAurB) has been preliminarily examined (Mon et al., 2014). In this study, we cloned the full-length cDNA of silkworm Aurora B kinase gene (referred to hereafter as *BmAurB*) and analyzed its phylogenetic relationship with Aurora kinases from other species. The spatio-temporal expression of the *BmAurB* gene during silkworm larval development was also profiled. We finally investigated the roles of BmAurB kinase in silkworm cells by RNA interference (RNAi) or inhibitor treatment.

2. Materials and methods

2.1. Full-length cDNA cloning of the BmAurB gene

The full-length cDNA sequence of the *BmAurB* gene was cloned through 5′ and 3′ rapid amplification of cDNA end (5′ and 3′ RACE) by using 5′-full RACE kit and 3′-full RACE core set (Takara, Japan). In brief, the silkworm *Dazao* strain was reared on fresh mulberry leaves at 25 °C under 12-hour light/12-hour dark cycle. Total RNA extracted from silkworm ovaries was reversely transcribed into cDNA template. Based on the predicted silkworm homolog of *Drosophila* Aurora B kinase and according to the instructions of RACE Kits, we coloned the core fragment, 5′ upstream, and 3′ downstream regions of the *BmAurB* gene, and subsequently performed DNA sequencing and full-length cDNA assembling. The primers are listed in Supplementary Table 1. The online CDD search program (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) was used to characterize domain architecture of the BmAurB protein.

2.2. Phylogenetic analysis

An online BLAST search in NCBI database was performed to identify Aurora kinases in human, mouse (*Mus musculus*), the fruit fly (Diptera), the honey bee (*Apis mellifera*, Hymenoptera), red flour beetle (*Tribolium castaneum*, Coleoptera), monarch butterfly (*Danaus plexippus*, Lepidoptera), and malaria mosquito (*Anopheles gambiae*, Diptera). The amino acid sequences for the S_TKc domains of the BmAurB protein and other identified Aurora kinases were used to perform a multiple sequence alignment by using ClustalX program (Larkin et al., 2007). A neighbor-joining phylogenetic tree with a bootstrap of 1000 replicates was built based on the alignment result and was refined by MEGA4 program (Tamura et al., 2007).

2.3. RT-PCR assay

RT-PCR examination was carried out to determine the expression of the *BmAurB* gene in multiple tissues of silkworm larvae on day 3 of the fifth instar (L5D3), in brain and silk gland during silkworm larval development, and in silkworm ovary derived BmN4-SID1 cells following RNAi experiment. RNA extraction, cDNA synthesis, and PCR reaction were performed as previously described (Cheng et al., 2008; Meng et al., 2015). The silkworm eukaryotic translation initiation factor 4A (*eIF-4a*) gene and actin protein A3 (*Actin 3*) gene were used as the controls for quantitative and semi-quantitative RT-PCR experiments, respectively. The primers are listed in Supplementary Table S1.

2.4. RNAi and inhibitor-mediated inhibition of the BmAurB gene in silkworm BmN4-SID1 cells

The silkworm ovary derived BmN4-SID1 cells were cultured in IPL-41 medium (Gibco) supplemented with 10% serum at 27 °C. The dsRNAs targeting *BmAurB* gene and *dsRed* gene (control) were separately synthesized to perform RNAi-mediated knockdown. The related primers are listed in Supplementary Table 1. The BmN4-SID1 cells were seeded into 24-well plate with 10^5 cells per well in 0.5 mL medium and were cultured for 12 h. After treating by dsRNAs (a dosage of 2 μg per well) for 5 days or by AZD1152-HQPA (Sigma) as Aurora B inhibitor (a dosage of 1 uM per well) for 3 days, these cells were collected to check cell cycle progression via flow cytometry analysis and immunostaining. DMSO treatment was used as a control of inhibitor-mediated inhibition.

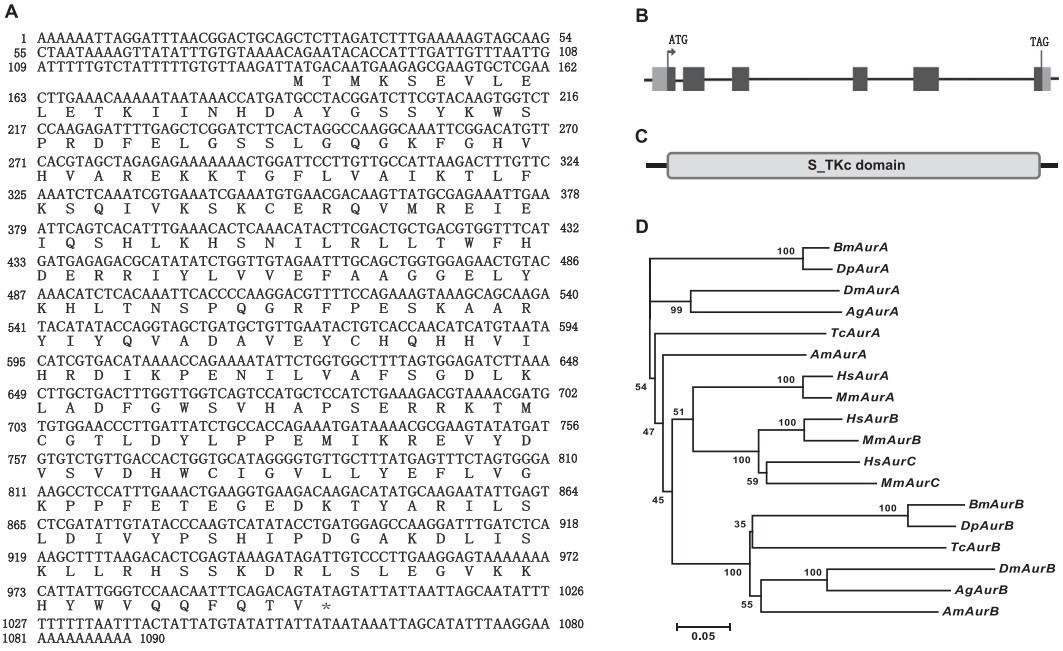

Fig. 1. Identification and phylogenetic analysis of the *BmAurB gene*. (A) The nucleotide acid and deduced amino acid sequence of the full-length cDNA for the *BmAurB* gene. The stop codon is marked by an asterisk. (B) Exon-intron structure of the *BmAurB* gene. The exon and intron are indicated by the rectangle and line, respectively. (C) Functional domain prediction of the BmAurB protein. (D) Phylogenetic tree of aurora kinases from silkworm and other surveyed species. This tree was built based on the multiple alignments of amino acid sequences for the S_TKc domains of BmAurB and other aurora kinases. Bm, *Bombyx mori*; Dm: *Drosophila melanogaster*; DP, *Danaus plexippus*; Ag, *Anopheles gambiae*; Am, *Apis mellifera*; Tc, *Tribolium castaneum*; Hs, *Homo sapiens*; Mm, *Mus musculus*.

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