Comparative Biochemistry and Physiology, Part A 157 (2010) 237–245

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Identification and dynamic transcription of KIF3A homologue gene in spermiogenesis of *Octopus tankahkeei*

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ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 1 July 2010
Accepted 2 July 2010
Available online 17 July 2010

Keywords:
kif3a
Kinesin-II
Spermiogenesis
Octopus

ABSTRACT

KIF3A is a subunit of the heterotrimeric Kinesin-II motor which achieves fame for its pivotal roles in the assembly and maintenance of cilia and flagella and in intracellular transport of membrane bound organelles and protein complexes in various tissues. Its intimacy to the cell's antenna, namely the primary cilia, makes it also involved in some signaling transduction pathways. To test the idea that KIF3A functions during spermiogenesis of the octopod *Octopus tankahkeei*, we hereby identified a gene (designated as *ot-kif3a*) encoding a protein apparently homologous to a group of KIF3As, from the testis of this organism. The full-length *ot-kif3a* comprised a 344 bp 5' untranslated region, a 2241 bp open reading frame and a 147 bp 3' untranslated region. The putative protein consisted of 746 amino acid residues with a calculated molecular weight of 85 kDa and a predicted isoelectric point of 6.36. It shared an overall sequence identity of 69%, 69%, 69% and 67% to KIF3A from *Homo sapiens*, *Rattus norvegicus*, *Mus musculus* and *Danio rerio*, respectively. Tissue distribution profile analysis unraveled its presence in all the tissues examined. *In situ* hybridization of mRNA in spermionenic cells demonstrated that *ot-kif3a* was expressed moderately at the beginning of spermiogenesis. The abundance of transcripts increased in intermediate spermatid and peaked in drastically remodeling and final spermatids. In mature sperm, the message was still visible in the head and tail. The temporal and spatial expression dynamics of *ot-kif3a* during spermiogenesis supports the possibility that the putative motor protein, OtKIF3A, participates in the major cytological events during this differentiation program and is vital for the acquisition of the final cellular phenotype.

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1. Introduction

Spermiogenesis is the differentiation of haploid spermatids into highly condensed mature spermatozoa and brings about remarkable cellular and morphological changes occurring to a spermatid. These changes depend on intracellular targeting of various materials at specific times and places (Navolanic and Sperry, 2000), dramatic remodeling of some organelles (Hess and Renato, 2008), and intercellular communication between different cells in testis (Mruk and Cheng, 2004; Ruwanpura et al, 2010). Among all the cytological changes during spermiogenesis, acrosome biogenesis, nuclear reshaping and flagellum formation are the most prominent and crucial events inviting tremendous interests from researchers (Kierszenbaum and Tres, 2004; Escalier, 2003; Yang and Sperry, 2003). Nonetheless, the underlying mechanism directly governing these events still lacks adequate understanding.

There is an increasingly stronger voice claiming that cytoskeleton network and molecular motors, including microtubule-associated kinesin and dynein and actin-associated myosin, are involved in many ways in differentiation programs such as spermiogenesis (Sperry and Zhao, 1996; Miller et al., 1999; Guttman et al., 2000; Navolanic and Sperry, 2000; Chennathukuzhi et al., 2003; Vaid et al., 2007; Wang and Sperry, 2008). Molecular motors are responsible for sorting and translocation of different materials within a cell. The integral activity of molecular motors also has the potential of providing direct force for shaping of major organelles vital for the acquisition of the final cellular phenotype (Navolanic and Sperry, 2000; Wang et al., 2010). In addition, some motor proteins may even have roles in communication between different cells and regulation of gene transcription through intervention in some signaling pathways (Sisson et al., 1997; Hogeveen and Sassone-Corsi, 2006)).

Kinesin is one type of motor proteins studied extensively in a wide range of organisms and tissues (Hirokawa and Takemura, 2004; Hirokawa et al., 2009). KIF3A is an important member of a kinesin superfamily named the Kinesin-II family or KIF3 family (Cole et al.,

Abbreviations: KIF3A, kinesin family member 3A; RT-PCR, reverse transcription polymerase chain reaction; cDNA, DNA complementary to RNA; RACE, rapid amplification of cDNA ends; ORF, open reading frame; UTR, untranslated region(s); PFA-PBS, paraformaldehyde in phosphate buffered saline; DIG, digoxigenin; ISH, *in situ* hybridization.

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doi:10.1016/j.cbpa.2010.07.004

1992, 1993; Yamazaki et al., 1996). Kinesin-II motor is a heterotrimeric complex assembled by KIF3A, either KIF3B or KIF3C, and an accessory subunit, KAP3 (Kondo et al., 1994; Yamazaki et al., 1995; Wedaman et al, 1996; Takeda et al., 2000). This motor plays pivotal roles in intraflagellar transport (IFT) (Marszalek and Goldstein, 2000; Rosenbaum and Witman, 2002; Cole, 2005; Scholey, 2008) and is indispensable for the assembly and maintenance of cilium and flagellum (Morris and Scholey, 1997; Corbit et al., 2008; Pedersen et al., 2008). It also functions as an anterograde motor for fast axonal transport of membranous organelles including synaptic vesicle, mitochondria and precursor of axon plasma membrane (Coy and Howard, 1994; Kondo et al., 1994). Other researches evidence that primary cilium works as eukaryotic cell's antenna transducing a multitude of sensory stimuli to coordinate a number of physiological and developmental signaling pathways (Marshall and Nonaka, 2006; Singla and Reiter, 2006; Pedersen and Rosenbaum, 2008). Because of its intimacy to the primary cilium, KIF3A is also implicated in some signaling pathways such as Hedgehog and Wnt pathways (Corbit et al., 2008; Ocbina and Anderson, 2008) and might control embryonic body planning, particularly for determination of left–right asymmetry (Takeda et al., 1999) and patterning of vertebrate skeleton (Kolpakova-Hart et al., 2007).

Spermiogenesis of the octopod *Octopus tankahkeei* is a good model system to study the involvement of kinesin in cytological changes because of the dramatic nature of these changes in this organism (Li and Dong, 2003; Zhu et al., 2005, 2006; Li et al., 2010; Wang et al., 2010; Yu et al., 2010). Preciously, we identified a gene encoding a protein homologous to rat KIFC1 (Wang et al., 2010), a C-terminal retrograde motor protein potentially responsible for vesicles transport from Golgi to acrosome and reshaping of nucleus (Yang and Sperry, 2003; Yang et al., 2006). Among other cytological transformations during spermiogenesis of *O. tankahkeei*, generation and function of the slender flagellum and the transient activity of a manchette-like perinuclear complex seem also likely to require commitment of anterograde motors such as Kinesin-II. We hypothesize that the octopus counterpart of rat Kinesin-II will take part in the assembly and maintenance of spermatic flagellum, intraflagellar transport and intramanchette transport, and primary cilia based signal transduction. In the current study, we cloned a cDNA corresponding to a polypeptide highly related to KIF3As from other species. We also examined its tissue distribution profile and determined its temporal and spatial expression pattern during spermiogenesis. At last, the functional implications for this motor from these data were discussed and anatomized on the basis of our hypothesis.

2. Materials and methods

2.1. Sampling

Specimens of *O. tankahkeei* were purchased from Ningbo Aquatic Products Market from December, 2008 to November, 2009. Twenty adult male individuals were selected and transported to the Sperm Laboratory at Zhejiang University in sea water tanks with aeration facility. Following temporary maintenance, the animals were anesthetized on ice and dissected to obtain the testis, hepatopancreas, muscle, gill, ink sac and intestine. The tissues were then swiftly placed and preserved in liquid nitrogen for RNA extraction. Testes and spermatophore sacs from six different animals were also subjected to fixation with 4% PFA-PBS (pH 7.4) for *in situ* hybridization.

2.2. Degenerate primer design and RT-PCR

Protein sequences of KIF3A homologues from various species were aligned by Clustal W software online (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and a number of conserved regions of protein were detected. One pair of degenerate primers, F-A1 and R-A1, was designed using the online tool CODEHOP (http://bioinformatics.weizmann.ac.il/blocks/codehop.html) based on the amino acid residues of two selected conserved regions.

Total RNA from testis was extracted according to established protocols in our laboratory (Wang et al., 2010). Bioready First-strand Synthesis Kit (Bioer) was used to have the mRNA reverse transcribed and the resulting cDNA was subjected to PCR with degenerate primers (F-A1/R-A1) in order to probe a fragment of the target gene. The exact cycling parameters of touchdown PCR were as follows: 94 °C for 3 min; 12 cycles of 94 °C for 30 s, 55 °C (which was reduced by 0.5 °C per cycle) for 30 s and 72 °C for 30 s; 23 cycles of 94 °C for 30 s, 49 °C for 30 s and 72 °C for 30 s; 72 °C for 10 min. The PCR product was gel-excised, ligated to PMD-18 T vector (Takara), transformed into DH5α Competent cells (Takara) and finally sequenced by Shanghai Sangon Company.

2.3. Rapid amplification of cDNA ends (RACE)

After a portion of the target cDNA was obtained, rapid amplification of cDNA ends was performed with SMART RACE Kit (Clonetech). Two gene specific primers (RACE-F and RACE-R) designed from the obtained sequence were coupled with the 3' and 5' adaptor primers provided in the kit, respectively, and used in 3' RACE-PCR and 5' RACE-PCR. Reverse transcription and adaptor ligation were completed using reagents provided in the kit. The reaction mixture composition and cycling conditions for RACE-PCR were established as recommended in the manufacture's instruction. All PCR products were subjected to transformation and sequencing mentioned above. Finally, the full-length cDNA was compiled by overlapping the 5' RACE fragment, the degenerate PCR derived fragment and the 3' RACE fragment.

2.4. Tissue expression pattern analysis

To examine tissue distribution pattern of this gene, RNA with the same quantity from the muscle, hepatopancreas, testis, gill, ink sac and intestine of adult males underwent RT-PCR, respectively, with a pair of gene specific primers (F and T) resulting in a 320 bp product. Primers of Octopus β-actin, Actin-F and Actin-R were used in parallel experiments as a positive control. Each experiment had at least three replications. The exact conditions of PCR steps were fixed as: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 55 °C for 20 s and 72 °C for 30 s; 72 °C for 10 min. All primer sequences were presented in Table 1.

2.5. In situ hybridization

In situ hybridization was carried out according to the same protocol detailed in our previous study (Wang et al., 2010). Briefly, an 847 bp fragment of *ot-kif3a* was produced by PCR using a pair of gene specific primers (Temp-F and Temp-R) and ligated to PGEM-T EASY Vector (Promega). Then, DIG-11-UTP incorporated riboprobe complementary

Table 1
A summary of primers used in the study.

Primer name	Sequence	Purpose
F-A1	TTCGCTTACGGNCARACNGG	Degenerate PCR
R-A1	CTTTCAGAACCAGCNARRTCNAC	
RACE-F	AGGTTTACGGACAACACTCG	3′ RACE
RACE-R	CGTAGACACCAACATCGGGCCGTTC	5′ RACE
F	TATGGAAGGTTTACGGACAA	Tissue distribution analysis of *ot-kif3a*
R	ATGGGCATTCATATTGGTAG	
Temp-F	TAAGGTACGCTAACCGTGCTAAG	*In situ* hybridization
Temp-R	AATTGCCATTCACCTATGTCATC	
Actin-F	AGCACCCTGTCTTATTGACTGAG	β-actin primers for positive control
Actin-R	TCACGACCAGCCAAATCAAGACG	

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