Animal Reproduction Science 184 (2017) 59–77

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Dynamic transcription and expression patterns of KIF3A and KIF3B genes during spermiogenesis in the shrimp, *Palaemon carincauda*

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ARTICLE INFO

Keywords:
Acrosome formation
KIF3A/3B
Nucleus shaping
Palaemon carincauda
Spermiogenesis

ABSTRACT

Spermiogenesis is a highly ordered and complex process in the male germ cell differentiation. The microtubule-based motor proteins KIF3A and KIF3B are required for the progression of the stages of spermiogenesis. In this study, the main goal was to determine whether KIF3A and KIF3B have a key role in spermiogenesis in *Palaemon carincauda.* The complete cDNA of KIF3A/3B from the testis of *P. carincauda* was cloned by using PCR and rapid amplification of cDNA ends (RACE). The predicted secondary and tertiary structures of KIF3A/3B contained three domains which were the: a) head region, b) stalk region, and c) tail region. Real-time quantitative PCR (qPCR) results revealed that KIF3A and KIF3B mRNAs were obtained for all the tissues examined, with the greatest gene expression in the testis. *In situ* hybridization indicated the KIF3A and KIF3B mRNAs were distributed in the periphery of the nuclear in the early spermatid of spermiogenesis. In the middle and late spermatid stages, KIF3A and KIF3B mRNAs were gradually upregulated and assembled to one side where acrosome biogenesis begins. In the mature sperm, KIF3A and KIF3B mRNAs were distributed in the acrosome cap and spike. Immunofluorescence studies indicated that KIF3A, tubulin, mitochondria, and Golgi were co-localized in different stages during spermiogenesis in *P. carincauda*. The temporal and spatial gene expression dynamics of KIF3A/3B indicate that KIF3A and KIF3B proteins may be involved in acrosome formation and nucleus shaping. Moreover, these proteins can transport the mitochondria and Golgi that facilitate acrosome formation in *P. carincauda*.

1. Introduction

Spermiogenesis is a complex process that brings about marked cellular and morphological changes through three significant developmental stages (i.e., nuclear shaping, acrosome biogenesis, and flagellum formation; Wang and Sperry, 2008; Hermo et al., 2010). These stages are, in essence, strictly regulated by stepwise signaling pathways and stage-specific gene expression (Kimmins et al., 2004 Hogeveen and Sassone-Corsi, 2006; Rajkovic et al., 2010). The kinesin superfamily proteins (KIFs) represent a group of

Abbreviations: UTR, untranslated region; RACE, rapid amplification of cDNA ends; qPCR, real-time quantitative polymerase chain reaction; ORF, open reading frame; IFT, intraflagellar transport; KIFs, kinesin superfamily proteins; LCx, lamellar complex; AFS, acroframosome; KAP3, kinesin-associated protein 3; PBS, phosphate buffered saline; OCT, optimum cutting temperature; DEPC, diethylpyrocarbonate; SSC, sodium chloride; DIG, digoxigenin; PFA, paraformaldehyde; BSA, albumin from bovine serum; TEM, transmission electron microscopy; SD, standard deviation

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http://dx.doi.org/10.1016/j.anireprosci.2017.06.017
Received 23 November 2016; Received in revised form 29 May 2017; Accepted 22 June 2017
Available online 01 July 2017
0378-4320/

molecular motors that transport various intracellular materials and create remodeling of some organelles in the testis (Navolanic and Sperry, 2000; Hirokawa and Takemura, 2004; Hess and Renato, 2008).

Kinesin-II is an important member of the KIFs and typically a component of N-terminal motor proteins that travel from the minus to the plus end of the microtubules (Hirokawa, 1998; Hirokawa et al., 1998). Previous studies of kinesin-II function have revealed that it transports opsin and arrestin from the inner photoreceptor to the outer segment, and deletion of KIF3A results in abnormal development of the photoreceptor outer segment and mis-localization of rhodopsin (Marszalek et al., 2000; Raghupathy et al., 2016). Moreover, kinesin-II complex can directly regulate GLI protein trafficking activity, and subsequently regulate the Hedgehog (HH) signaling pathway, which is necessary for embryonic development and also functions in adult tissue homeostasis, renewal, and regeneration (McMahon et al., 2003; Briscoe and Therond, 2013; Carpenter et al., 2015). Many studies have indicated that deletion of KIF3A can cause cystic and neoplastic renal lesions, accelerate renal cyst formation, and inhibit epithelial-to-mesenchymal transition via suppression of the β-catenin-dependent Wnt signaling pathway (Lehmann et al., 2015; Guinot et al., 2016; Shan and Li, 2016; Wang et al., 2016).

Apart from the above-mentioned functions, the kinesin-II gene is also expressed in developing male germ cells and mature sperm in different species. Intraflagellar transport (IFT) is a bidirectional movement along the axoneme using motor complexes of kinesin-II and cytoplasmic dynein-2 (Baker et al., 2003; Pan et al., 2006; Lehti and Sironen, 2016). Many studies indicate that KIF3A/3B and IFT are required to install and maintain the flagellum in mammals, cephalopods, and amphibians (Wang et al., 2010; Dang et al., 2012; Hu et al., 2012; Lehti et al., 2013). Another report states that KIF3A/3B may participate in the transport vesicles, which may also be responsible for acrosome biogenesis and nuclear reshaping (Lu et al., 2014). Lehti et al. (2013) demonstrated that depletion of KIF3A results in a malformed head shape and defects in sperm tail formation. Although KIF3A/3B has a pivotal role in many developmental stages of spermiogenesis, its mechanisms of acrosome formation have not reported, and the functions of kinesin-II remain unknown during spermiogenesis in *P. carincauda*.

P. carincauda is a commercially important aquaculture species, which inhabits the coastal areas of China. In *P. carincauda*, no flagellum is formed, and the sperm structure resembles an inversed umbrella, comprising a main body, an acrosome cap, and a spike (Zhang and Yang, 2003). The lamellar complex (LCx) is an important structures with regard to acrosome formation during spermiogenesis in *P. carincauda* (Zhang and Yang, 2003; Hou and Yang, 2013). The LCx consists of the Golgi complex, endoplasmic reticulum, mitochondria, lysosomes, and centriole (Yang, 1998). Moreover, the LCx also elongates gradually to form the proacrosome, which eventually forms the acrosome cap and spike (Li et al., 2010). Many studies indicate that KIF3A/3B has a key role in many events during spermiogenesis. It is, therefore, important to study the function of KIF3A/3B during spermiogenesis in *P. carincauda*. In the current study, complete cDNA sequences of KIF3A and KIF3B were cloned. The tissue distribution profile was analyzed and it was determined that there was a temporal and spatial gene expression pattern during spermiogenesis in *P. carincauda*. It is hypothesized that the *P. carincauda* counterpart of kinesin-II transports the LCx components, which facilitates acrosome formation and nuclear shape during spermiogenesis in *P. carincauda*. The primary goal of the present research was to further elucidate the mediated mechanisms and the functions of kinesin-II during spermiogenesis in *P. carincauda*.

2. Materials and methods

2.1. Animals and sampling

Adult *P. carincauda* that developed in their natural habitat were purchased from Ningbo Aquatic Products Market (Ningbo, China). The zoetic individuals were dissected immediately and the gill, testis, muscle, hepatopancreas, and heart were excised. The dissected tissues were placed in liquid nitrogen immediately, and stored at −80 °C until used for total RNA and protein extraction. In addition, the testis was fixed with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS; pH 7.4) for *in situ* hybridization and immunofluorescence. The Institutional Animal Care and Use Committee at the Zhejiang Laboratory Animal Research Center and Ningbo University approved the management of animals used in the present study.

2.2. RNA extraction and reverse transcription

The TriZol reagent (Tiangen, Beijing, China) was used to extract total RNA from the hepatopancreas, gill, testis, muscle, and heart of *P. carincauda*. The BioRT cDNA First Strand Synthesis Kit (Takara, Dalian, China) was used for ordinary reverse transcription assays. In addition, the Smart RACE cDNA Amplification Kit (CloneTech, Mountain View, USA) and 3′ full RACE Amplification Kit (Takara) was used for 5′ RACE and 3′ RACE reverse transcription assays. The reverse transcription products were stored at −20 °C for future PCR.

2.3. Full-length cDNA cloning

Primers (Table 1) were designed by using the Primer Premier 5 software to obtain the intermediate segment sequences of KIF3A and KIF3B. The PCR was used to amplify the intermediate segment sequence. The PCR programs were conducted as follows: 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s; and 72 °C for 10 min for the final extension. The PCR products were separated by agarose electrophoresis and the bands visualized using Gelview (Bioteke, Beijing, China). The expected bands were extracted and purified using Quick-type DNA Gel Extraction Kit (Bioteke). The purified fragments were ligated to PMD-18T vector (Takara), propagated into competent cells (Escherichia coli DH5α), and ultimately sequenced by Beijing Genomics Istitute (Beijing,