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Novel localization of Aurora A kinase in mouse testis suggests multiple roles in spermatogenesis

Marquita L. Johnson, Rong Wang, Ann O. Sperry*

Anatomy and Cell Biology, East Carolina University, Brody School of Medicine, 600 Moye Blvd, Greenville, NC, 27834, USA

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ABSTRACT

Male germ cells are transformed from undifferentiated stem cells into spermatozoa through a series of highly regulated steps together termed spermatogenesis. Spermatogonial stem cells undergo mitosis and differentiation followed by two rounds of meiotic division and then proceed through a series of dramatic cell shape changes to form highly differentiated spermatozoa. Using indirect immunofluorescence, we investigated a role for the mitotic kinase, Aurora A (AURKA), in these events through localization of this protein in mouse testis and spermatozoa. AURKA is expressed in several cell types in the testis. Spermatogonia and spermatocytes express AURKA as expected based on the known role of this kinase in cell division. Surprisingly, we also found AURKA localized to spermatids and the flagellum of spermatozoa. Total AURKA and activated AURKA are expressed in different compartments of the sperm flagellum with total AURKA found in the principal piece and its phosphorylated and activated form found in the sperm midpiece. In addition, active AURKA is enriched in the flagellum of motile sperm isolated from cauda epididymis. These results provide evidence for a unique role for AURKA in spermatogenesis and sperm motility. Defining the signaling mechanisms that govern spermatogenesis and sperm cell function is crucial to understanding and treating male infertility as well as for development of new contraceptive strategies.

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1. Introduction

Spermatogenesis is a complex and tightly regulated developmental process that transforms undifferentiated spermatogonia into streamlined, motile sperm capable of fertilization. Precise control of spermatogenesis is critical to produce viable sperm that are capable of fertilizing an egg to ensure overall propagation of the species. Spermatogenesis begins with a series of mitotic events to renew the spermatonial stem cell population and produce progeny that initiate differentiation, followed by two meiotic divisions to produce haploid spermatids. A series of striking morphological changes follows whereby male germ cells achieve their final form composed of condensed chromatin packaged into the head, an associated acrosome responsible for egg penetration, and a flagellum to propel the sperm towards the egg.

It is remarkable that much of spermatogenesis occurs in the absence of ongoing transcription and therefore must rely on post-translational modifications (PTMs) to regulate this process. Existing sperm proteins are subject to a wide range of PTMs to modulate their activity including phosphorylation, acetylation, glycylation, and sumoylation [1,2]. While well-defined signaling pathways such as MAPK and others are critical for spermatogenesis [3,4]; this complex developmental program likely involves a unique and varied array of phosphorylation/dephosphorylation pathways to regulate differentiation. Here we describe our discovery of a novel localization for a mitotic kinase, Aurora A (AURKA), in spermatids and spermatozoa.

The family of Aurora kinases (AURKA, AURKB, and AURKC) are a related family of serine-threonine kinases with critical but distinct roles in cell division in somatic and germ cells [5]. AURKA has well-established roles in formation and function of the mitotic spindle. AURKA localizes to the centrosome and spindle fibers and is essential for centrosome maturation, separation, mitotic entry, and bipolar spindle assembly [6]. AURKB is a DNA passenger protein that functions in spindle checkpoint activation and is associated with kinetochores from prophase to anaphase and then is transported to the spindle midzone at telophase [7]. AURKC is expressed primarily in the testis with proposed roles in meiosis [8–10].

* Corresponding author.
E-mail addresses: johnsonmarq15@ecu.edu (M.L. Johnson), wangr@ecu.edu (R. Wang), sperrya@ecu.edu (A.O. Sperry).

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In this work we describe a novel localization for AURKA during spermatogenesis. In addition to being found in dividing spermatogonia and spermatocytes, as predicted by its function in dividing cells, we localized AURKA to developing spermatids and spermatozoa. The localization of AURKA in spermatids and spermatozoa has not been described previously and suggests distinctive roles for this kinase in sperm development and motility.

2. Methods and materials

2.1. Animals

Adult male CD1 mice were purchased from Charles River Laboratories (Raleigh, NC). All use of animals was approved and conducted in accordance with International Animal Care and Use Committee (IACUC) of East Carolina University, protocol #W179f.

2.2. Immunoprecipitation

Mouse testes were collected then homogenized in lysis buffer (10 mM Tris-HCl pH 7.0, 1 mM EGTA, 1 mM EDTA 10 mM benzamidine, 1% NP-40) containing 0.1% BME, 1% PMSF and 1% protease inhibitor cocktail. Lysates corresponding to 350 μm total protein were centrifuged twice at 14,000×*g* at 4 °C for 30 min each. Lysates were precleared with 30 μl of Protein G Sepharose beads (Bio-Vision; Milpitas, CA) by rotating for 1 h at 4 °C. The clarified lysates were then collected following centrifugation and complexes formed by rotating the supernatant overnight at 4 °C with 2 μg of AURKA antibody (pA5-32035, Thermo-Fisher Scientific; Waltham, MA). Immune complexes were captured by rotating the sample in 30 μl of beads for 1 h at 4 °C. Beads were washed five times in lysis buffer, denatured by boiling in 2× sample buffer for 5 min and proteins analyzed by SDS-PAGE followed by blotting with rabbit anti-total AURKA (1:250 pA5-32035, Thermo-Fisher Scientific; Waltham, MA) and Veriblot anti-rabbit HRP (1:2500; Abcam; Cambridge, MA). The negative control was precleared lysate without antibody.

2.3. Indirect immunofluorescence

Adult mouse testes were incubated in 4% paraformaldehyde (PFA) for 24 h, then placed in 30% sucrose for 24–48 h. Frozen tissues were sliced into 10 μm sections, blocked in 3% BSA for 1 h at room temperature, and then incubated with anti-Aurora A antibody (IHC, 1:100; Bethyl Laboratories; Montgomery, TX) overnight at 4 °C. Sections were washed in TBST 3 times at room temperature for 5 min each, then incubated in secondary Alexa Fluor 594 antibody (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA.). Vectashield with DAPI was used to visualize DNA (Vector Laboratories Inc; Burlingame, CA).

Freshly excised epididymides were carefully disassociated with forceps and separated into caput, corpus and cauda sections and placed in pre-warmed phosphate buffered solution and 4–6 cuts were made in each section of the epididymis. Sperm were allowed to swim out for 10 min at 37 °C and the resulting sperm suspension was smeared onto glass slides for staining. Sperm density was verified under a light microscope then air-dried for at least 30 min at room temperature. Once completely dry, sperm were fixed in cold 4% PFA for 10 min then stored at 4 °C until further use. Before immunostaining, TBST was used to remove any residual PFA. Sperm were blocked in 3% BSA for 1 h at room temperature then stained with anti-Aurora A antibody (IHC, 1:50; Bethyl Laboratories; Montgomery, TX) or anti-phosphorylated Aurora A (Thr288) (1:50; ThermoFisher; Rochester, NY) along with PNA-lectin conjugated to Alexa Fluor 488 (1:200; ThermoFisher; Rochester, NY) to visualize the acrosome. DAPI was used to visualize the nucleus.

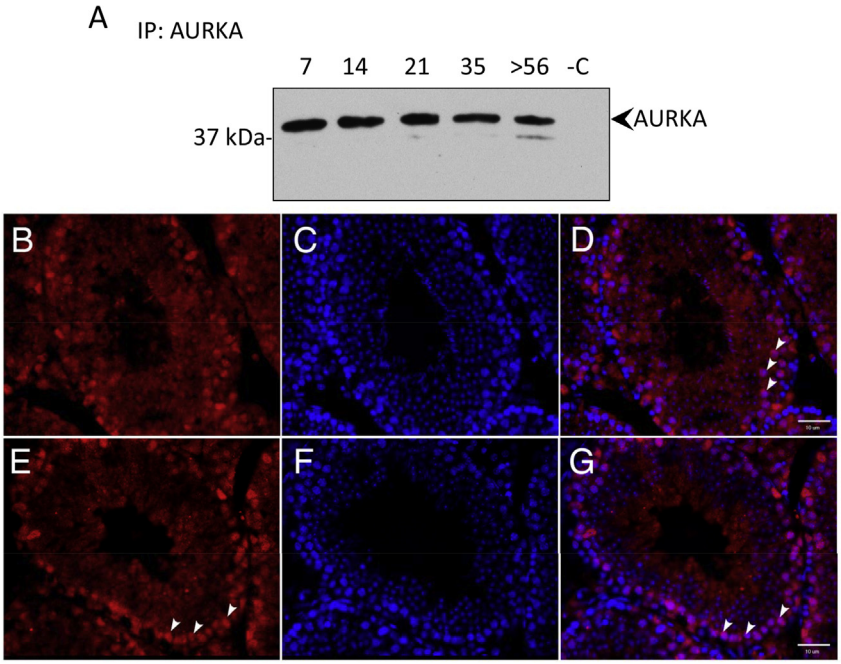

Fig. 1. Aurora A kinase is expressed in multiple male germ cell types in the testis.
(A) Protein lysates were prepared from testes of postnatal day 7, 14, 21, 35 and adult mice (>56 dpp). AURKA was precipitated with an AURKA specific antibody and detected with the same antibody. Proteins in lane -C where precipitated in the absence of antibody as control. AURKA expression was detected during the first wave of spermatogenesis in the testis when specific cell types appear at defined times after birth. **(B–G)** Frozen testes sections were stained with AURKA (red in **B** and **E**) and DNA with DAPI (blue in **C** and **F**) with the merged image in **D** and **G**. **(B–D)** AURKA localized to spermatogonia (representative spermatogonia indicated with arrowheads). **(E–G)** In other tubules, AURA localized to spermatocytes (representative spermatocytes indicated with arrowheads). Scale bars indicate 10 μm.

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