APPLICATION NOTE

N-STORM Super Resolution Microscope

Super-resolution Imaging of the Nanoscale Architecture of Sertoli Cell Cortical F-actin Network

Sperm, with its unique shape, is known to be generated from small spherical sperm cells in the seminiferous tubules of the testes. Previous research has shown that close interaction between sperm cells and Sertoli cells, which are supporting cells in seminiferous tubules, is important for normal sperm morphogenesis. Cell-to-cell adhesion between sperm cells and Sertoli cells consists of cell-to-cell adhesion molecules and cytoskeleton actin (Fig. 1); however, the structure of this F-actin and its formation/maintenance mechanism are mostly unknown, and their physiological significance is also unclear. This application note introduces an example in which Dr. Dean Thumkeo of the Department of Drug Discovery Medicine, Kyoto University Graduate School of Medicine clearly captured images of the architecture of a nanoscale three-dimensional network of cortical actin filaments in Sertoli cells using a super-resolution microscope.

Experiment Overview

Firstly, Dr. Thumkeo found that mDia1/3 double-knockout male mice are infertile. Next, he historically observed the sperm and seminiferous tubules, and found that the cause of male infertility was spermatogenesis abnormalities. Further, it was confirmed that both mDia1 and mDia3 were expressed at high levels in Sertoli cells. This revealed that the cause of spermatogenesis deficiency in mDia1/3 double-knockout mice was attributed to the Sertoli cell side rather than the sperm cell side.
In this study, actin filaments of primary cultured Sertoli cells were observed using the N-STORM super-resolution microscope with an XY axis resolution of about 20 nm and Z-axis resolution of about 50 nm in order to clarify the functions of mDia1/3 in Sertoli cells (Fig. 2).

The occupancy rate of the F-actin meshwork was calculated by segmenting the F-actin bundles in the obtained image and isolating them from the F-actin meshwork.
Also, in order to observe the dynamics of F-actin meshwork, actin filaments in primary cultured Sertoli cells were fluorescence labeled, and high-speed, high-resolution live imaging was performed with a spinning disc confocal microscope, and the polymerization rate of actin filaments constituting the F-actin meshwork was measured.
How mDia1 and mDia3 contribute to actin cytoskeleton structure and dynamics as well as spermatogenesis was studied using the above techniques.

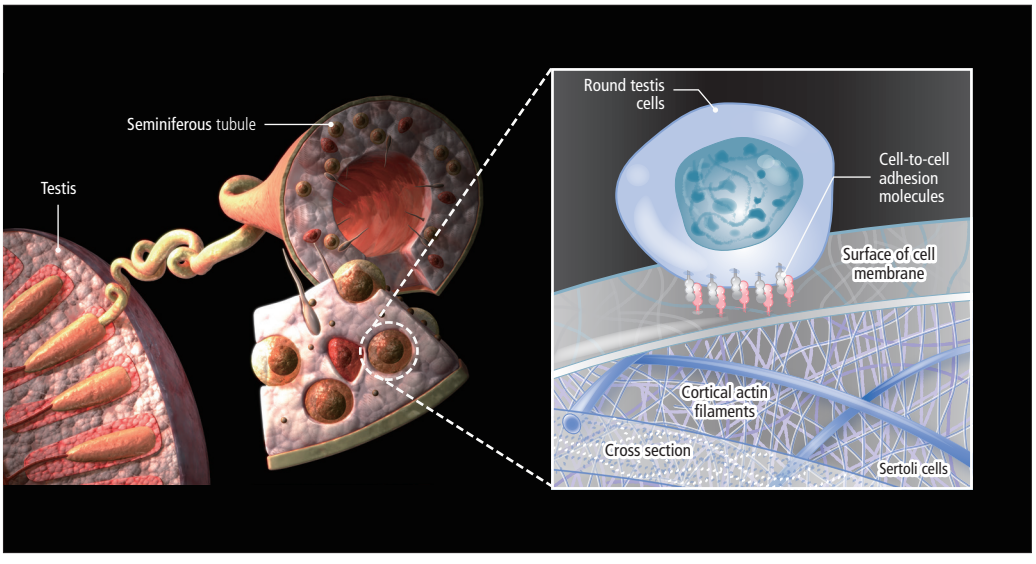

Fig. 1: Sertoli subcortical actin filaments generated by mDia1/3 are required for normal intercellular adhesion between Sertoli cells and round sperm cells, and are critical for sperm morphogenesis.

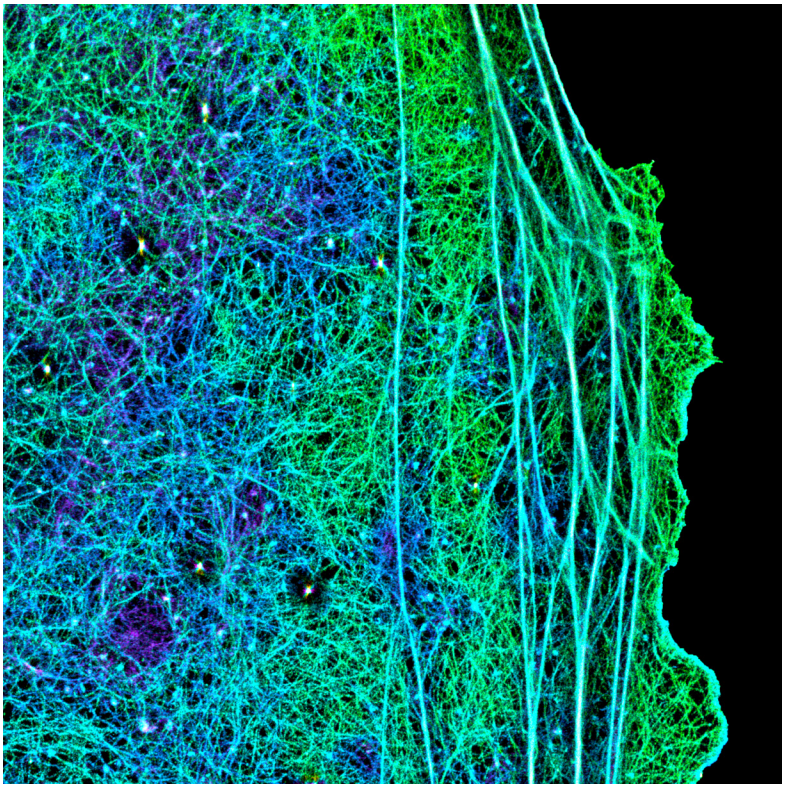

Fig. 2: Primary cultured cells of Sertoli cells derived from mouse testes were fixed with Glutaraldehyde, permeabilized with a buffer containing TritonX, and stained with Alexa Fluor 647-Phalloidin.
Pseudo color display of Z-axis depth of -300 to 300 nm
Objective: CFI Apochromat TIRF 100XC Oil

Results

TIRF 3D-N-STORM imaging using a super-resolution microscope revealed that there is a F-actin meshwork with a mesh size of about 100 nm immediately beneath the cell membrane of Sertoli cells, and that the density of this actin structure decreases significantly due to the double knockout of mDia1/3. In addition, confocal observation revealed a specific loss of actin filaments with a polymerization rate of 1.3 μm/s in mDia1/3 deficient cells. This is very similar to the actin polymerization rate of mDia3 calculated from single molecule imaging of mDia3. Consequently, mDia1/3 is considered a contributor to the formation and maintenance of F-actin meshworks.

Also, contractile actomyosin bundles are known to be important for the formation/maintaining of cell-to-cell adhesion from previous studies using cultured cells. In this study, from analysis with the N-STORM super-resolution microscope, actin filaments constituting F-actin meshwork in primary cultured Sertoli cells were found to be continuous with contractile actomyosin bundles. However, such contractile actomyosin bundles were significantly reduced and, instead, noncontractile espin-associated F-actin bundles were increased in mDia1/3 double knockout Sertoli cells. These results suggest that the mDia1/3 double deficiency impairs the formation and maintenance of adhesion between Sertoli cells and sperm cells.

Summary

This study confirmed that mDia1/3 contributes to normal spermatogenesis by regulating polymerization of F-actin meshwork in Sertoli cells, producing continuous contractile actomyosin bundles and forming and maintaining adhesion between Sertoli cells and sperm cells. This suggests that abnormalities in the actin cytoskeleton structure in Sertoli cells can be one of the causes of male infertility.

The causes of about half of the spermatogenic disorders that account for most cases of male infertility are unknown. This study, which clarified one of the causes of male infertility, is expected to provide suggestions for the development of new treatments.

References:

mDia1/3 generate cortical F-actin meshwork in Sertoli cells that is continuous with contractile F-actin bundles and indispensable for spermatogenesis and male fertility.
Sakamoto S*, Thumkeo D*#, Ohta H, Zhang Z, Huang S, Kanchanawong P, Fuu T, Watanabe S, Shimada K, Fujihara Y, Yoshida M, Ikawa M, Watanabe N, Saitou M, Narumiya S#
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PLoS Biology 2018, 16(9), doi: 10.1371/journal.pbio.2004874

Product Information

N-STORM Super Resolution Microscope

The N-STORM utilizes a localization technique called STochastic Optical Reconstruction Microscopy (STORM) to achieve a resolution of ten times that of conventional light microscopes. It allows imaging of the structure of cell organelles on a molecular level.
- Lateral resolution: Approx. 20 nm
- Axial resolution: Approx. 50 nm