APPLICATION NOTE

N-STORM Super Resolution Microscope

Localization Imaging of HORMAD Proteins and Meiotic Cohesins

During meiotic prophase I homologous chromosomes undergo pairing/synapsis and paternal and maternal chromosomes exchange genetic information. It is known that HORMAD proteins (HORMAD1 and HORMAD2) localize along the unsynapsed chromosome axes at that time, and that they monitor homolog synapsis. However, the molecular mechanism by which HORMAD proteins, which do not contain a definitive DNA-binding domain, localize to the chromosomal axes is still unclear. This application note introduces the study that clarified the mechanism by which HORMAD1 localizes to chromosome axes, and its interactions with the meiotic cohesins RAD21L and REC8, this study being the result of joint research conducted by Dr. Yasuhiro FUJIWARA of the Laboratory of Pathology and Development, Institute for Quantitative Biosciences, The University of Tokyo, and Dr. Kei-ichiro ISHIGURO of the Institute of Molecular Embryology and Genetics, Kumamoto University.

Experiment Overview

Dr. Yasuhiro FUJIWARA et al. have focused on the chromatin dynamics and transcriptional regulation mechanism in germ cells, and are researching what functions they have for cell proliferation and differentiation/fertilization.
This study aims to clarify how HORMAD1, which promotes the recruitment of DNA double-strand break (DSB) factors such as SPO11 to chromosomal axes in early axis formation, and is a factor in monitoring homologous chromosome synapsis, is localized on chromosomal axes, and to reveal its molecular mechanism (Fig. 1).

The chromosome axis consists of meiotic cohesins (RAD21L and REC8) and synaptonemal complex (SYCP2 and SYCP3). Through the image acquisition using N-STORM super-resolution microscope (Fig.2), the work of Dr. Fujiwara et al. confirmed how these chromosomal structural proteins contribute to the loading of HORMAD1, which does not contain a definitive DNA-binding domain, onto the chromosome.

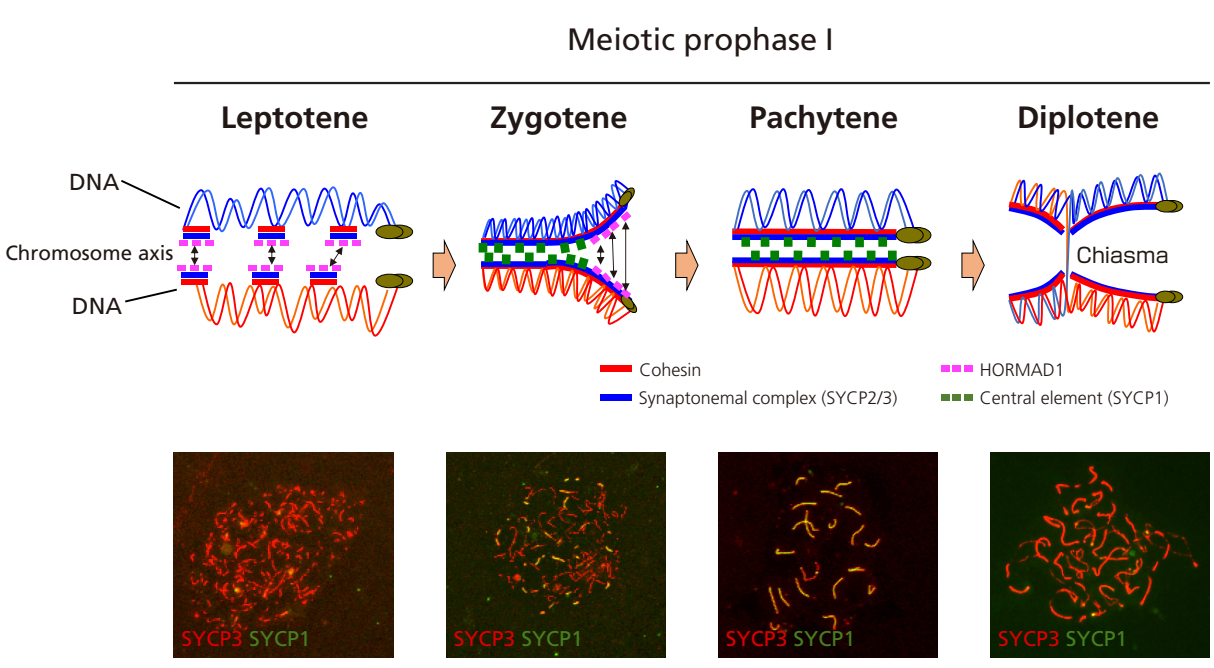

Fig. 1:
Meiosis progresses through DNA double-strand break, homologous recombination, homologous chromosome synapsis, crossing over, two meiotic divisions, etc., and most of this series of processes is done on the chromosomal axis. The mammalian chromosomal axis is composed of two factors called meiotic cohesin and synaptonemal complex. This axial structure begins to form with the onset of meiosis, and the localization of HORMAD1 there recruits the factors required for each event to the axis. This axial structure has a fine fragment shape immediately after the start of meiosis, then elongates as the formation of the axis progresses.

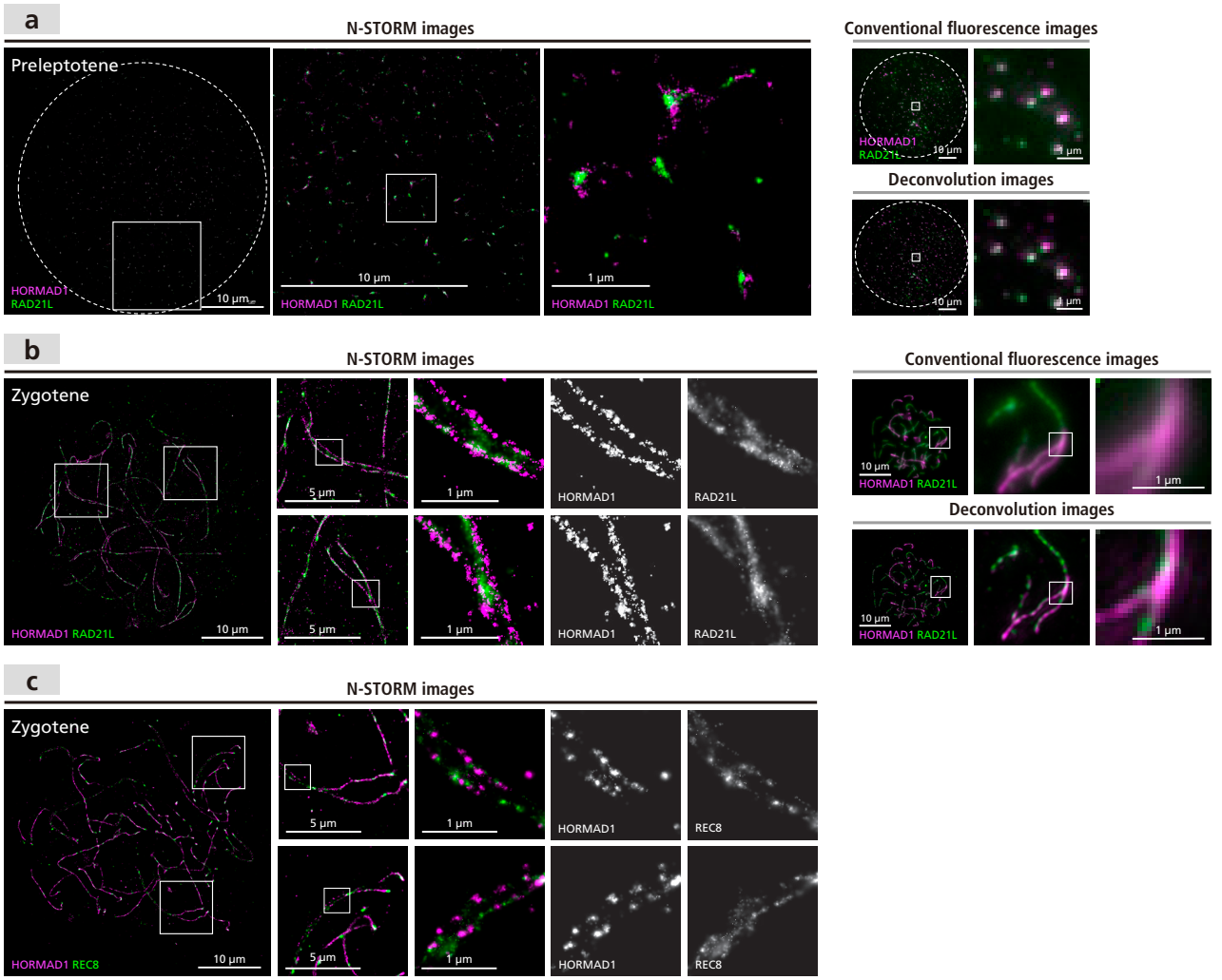

Fig. 2:
Mouse spermatocytes spread chromatin, in which HORMAD1 was labeled with CF 568 and RAD21L/REC8 with Alexa Fluor 647.
Juxtaposition in the preleptotene period, which is the initial stage of axis formation (a), and the localization of HORMAD1 and cohesin on the axial structure in the Zygotene period (b, c) could be confirmed more accurately with a super-resolution microscope than with a conventional fluorescence microscope. Thus, it can be seen that HORMAD1 is closely juxtaposed to RAD21L/REC8 enriched domains from the very early stages of the meiotic prophase I.
Objective: CFI SR HP Apochromat TIRF 100XC Oil

Results and Summary

In the early stage of axis formation in meiotic prophase I, HORMAD1, which monitors homolog synapsis, was localized very close to RAD21L and REC8 (Fig. 2a). From analysis of mice in which each factor was knocked out, it became clear that the localization of HORMAD1 on chromatin was mediated by meiotic cohesin prior to axis formation, and that the interaction between HORMAD1 and cohesin was partially supported by SYCP2.

Furthermore, it was clarified that HORMAD1 also shows localization patterns that are in part close to RAD21L and REC8 in the subsequent axis formation process (Fig. 2b, Fig. 2c), and that it contributes to the formation of the axis by coordinating with them.

Reference

Meiotic cohesins mediate initial loading of HORMAD1 to the chromosomes and coordinate SC formation during meiotic prophase.
Fujiwara Y., Horisawa-Takada Y., Inoue E., Tani N., Shibuya H., Fujimura S., Kariyazono R., Sakata T., Ohta K., Araki K., Okada Y., Ishiguro K.
PLOS Genetics (2020)16(9), e1009048

Product Information

N-STORM Super Resolution Microscope

This microscope employs STORM (STochastic Optical Reconstruction Microscopy), one of a number of localization methods, achieving about 10 times the resolution of conventional optical microscopes. This makes it possible to observe the structure of organelles at the single molecule level.

- Horizontal resolution: About 20 nm
- Z-axis resolution: About 50 nm