論文の内容の要旨

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論文題目

Novel protein MEIKIN regulates meiosis I specific kinetochore function in mouse

(新規タンパク質 MEIKIN はマウスの減数第一分裂特異的な動原体の機能を制御する)

1. Introduction

In mitosis, one round of DNA replication and chromosome segregation generates two daughter cells. During meiosis, however, one round of DNA replication followed by two sequential rounds of chromosome segregation, meiosis I and II, generate haploid cells (eggs or sperm). Therefore, the chromosome dynamics in meiosis differ in many ways from those of mitosis.

The kinetochore, a multi-protein structure that links chromosomes to the spindle microtubules, regulates chromosome segregation in mitosis and meiosis. Thus, the kinetochore is an essential apparatus for proper chromosome segregation. In mitosis, sister kinetochores, the pair of kinetochores on each sister chromatid, are captured by microtubules originating from both spindle poles (bi-orientation) and segregate to opposite poles to generate two identical daughter cells (equational division).

In meiosis I, unlike in mitosis, homologous chromosomes are physically connected by chiasmata, thus forming a bivalent. In addition, sister kinetochores are captured by microtubules originating from the same pole (mono-orientation) in metaphase I, and centromeric cohesion is protected during anaphase I (cohesion protection). Consequently, homologous chromosomes separate to opposite poles while sister chromatids move to the same pole during meiosis I (reductional division). Accordingly, the residual cohesion at the centromeres facilitates the

equational segregation of sister chromatids in meiosis II.

The important meiotic functions of kinetochores, mono-orientation and cohesion protection, are widely conserved in eukaryotic organisms. So far, meiosis-specific kinetochore proteins that regulate mono-orientation and cohesion protection are only reported in yeasts (Moa1 in fission yeast; Spo13 and Mam1 in budding yeast). However, their homologs have remained unidentified in other organisms including humans.

2. MEIKIN, a meiosis I specific novel kinetochore protein in mouse

Fission yeast Moa1 binds directly to Cnp3 (CENP-C, a conserved core component of centromeric chromatin) and localizes to kinetochores where it acts for mono-orientation and cohesion protection. In order to identify such meiosis specific kinetochore factors in mouse, Dr. Nambu in our lab performed yeast two hybrid screening to search mouse CENP-C binding proteins from a mouse testis cDNA library.

Our laboratory thereby identified a novel meiosis-specific kinetochore protein, MEIKIN (Meiosis-specific kinetochore protein). RT-PCR examination revealed that MEIKIN expression is germ cell-specific (in male testes and female ovaries) and not detected in other organs. Also, immunostaining of germ cells (male spermatocytes and female oocytes) confirmed that MEIKIN localizes to kinetochores from meiotic prophase I to metaphase I, but never appears in meiosis II or mitosis. We showed that conserved C-terminus sequences in MEIKIN play an essential role in localization at kinetochores and that MEIKIN is largely conserved among vertebrates including humans. Therefore, we conclude that MEIKIN is a novel meiosis-I-specific kinetochore protein in mammals.

3. MEIKIN regulates cohesion protection and mono-orientation

To elucidate the function of MEIKIN, Dr. Ishiguro in our lab and Dr. Takeda in Kumamoto Univ. generated MEIKIN knockout (KO) mice. MEIKIN KO male and female mice are both infertile although their development is normal. Although no histological abnormalities were observed in *Meikin*$^{-/-}$ testes and ovaries, approximately 2-fold increase in the kinetochore

number was observed in *Meikin*$^{-/-}$ round spermatids (post meiosis cells), which is indicative of meiotic cell division failure.

Intriguingly, *Meikin*$^{-/-}$ oocytes show premature sister chromatids separation at the onset of anaphase I, and chromosome alignment defects in metaphase II. Indeed, REC8, a protein mediating sister chromatid cohesion in meiosis, is lost in metaphase II chromosomes in *Meikin*$^{-/-}$ oocytes, suggesting that MEIKIN regulates REC8 protection during anaphase I. To explore this defect, we tested Shugoshin-2 (SGO2, centromeric cohesion protector in meiosis I) localization in *Meikin*$^{-/-}$ and found that SGO2 localization is indeed decreased in *Meikin*$^{-/-}$ oocytes. Taken together, these observations indicate that MEIKIN regulates REC8 protection during meiosis I, and partially stabilizes SGO2 localization in oocytes. Similar results were obtained in *Meikin*$^{-/-}$ spermatocytes.

To analyze the potential function of MEIKIN in mono-orientation, we measured the distance between sister kinetochore pairs in *Meikin*$^{-/-}$ oocytes, and observed an approximately 20% increase in prometaphase I. Next, we examined chromosome alignment by deleting MEIKIN in an *Mlh1*$^{-/-}$ background. Because homologous chromosomes are not linked by chiasmata in *Mlh1*$^{-/-}$, meiosis I cells accumulate univalents that fail to align on the metaphase I plate. Presumably, the property of tightly fused sister kinetochores prevents bi-orientation of univalents. Strikingly, in *Mlh1*$^{-/-}$ *Meikin*$^{-/-}$ oocytes, univalents are aligned and bi-oriented along the metaphase I spindle equator. Similar results were observed in *Meikin*$^{-/-}$ spermatocytes.

Therefore, we conclude that MEIKIN plays an important role in promoting mono-orientation in addition to protecting centromeric cohesion during mouse meiosis I.

4. MEIKIN associated PLK1 regulates meiosis I specific kinetochore function

To address the molecular function of MEIKIN, our laboratory (Dr. Nambu) performed yeast two hybrid screening of MEIKIN as bait from a mouse testis cDNA library. We identified Polo-like kinase PLK1 as a MEIKIN binding protein, and our laboratory (Dr. Ishiguro) confirmed this by immunoprecipitation-MASS spectrometry. We confirmed that PLK1 is indeed enriched to kinetochores, and, remarkably, that the PLK1 enrichment is largely impaired in

Meikin$^{-/-}$. These experiments reveal that PLK1 accumulation is in part dependent on MEIKIN during meiosis I.

To understand the function of PLK1 in kinetochore regulation, we inhibited PLK1 activity in meiosis I oocytes by adding BI 2536, a potent and selective inhibitor of PLK1. Surprisingly, transient BI 2536-treated wild type oocytes showed chromosome misalignment in metaphase II, implying that premature separation of sister chromatids occurs in anaphase I. Also, univalent alignment increased in BI 2536-treated *Mlh1−/−* oocytes as compared to non-treated *Mlh1−/−* oocytes. These experiments reveal that PLK1 activity is required for cohesion protection and the mono-orientation of sister kinetochores during meiosis I. We, therefore, conclude that MEIKIN regulates two important functions of meiotic kinetochores, cohesion protection and mono-orientation.

5. Conclusion

Here we identified a meiosis specific kinetochore protein, MEIKIN, in mouse that plays crucial roles in meiosis I. MEIKIN regulates two important functions of meiotic kinetochores, mono-orientation and cohesion protection, in part by stabilizing the localization of the centromeric cohesion protector Shugoshin-2. These functions are facilitated by the activity of Polo-like kinase PLK1, which is enriched to kinetochores dependent on MEIKIN.

MEIKIN is conserved in vertebrates including humans. Our group further revealed that MEIKIN is a functional homolog of fission yeast Moa1 and budding yeast Spo13. Therefore, it is reasonable to consider that the regulatory mechanisms of meiosis I specific kinetochore functions are conserved from yeast to mammals.

Published paper

Kim J.*, Ishiguro K.*, Nambu A., Akiyoshi B., Yokobayashi S., Kagami A., Ishiguro T., Pendas A. M., Takeda N., Sakakibara Y., Kitajima T. S., Tanno Y., Sakuno T. and Watanabe Y.
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“ Meikin is a conserved regulator of meiosis I specific kinetochore function.”
Nature, in press.