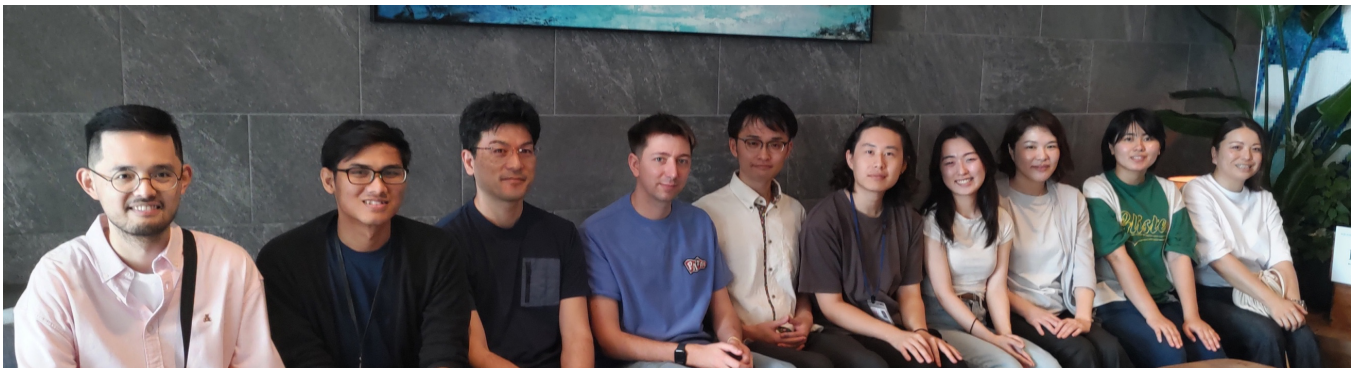
Cell Division Dynamics Unit

PI: Tomomi Kiyomitsu, Assistant Professor



(From left to right) Yang Ming, Muhammad Hamzah, Tomomi Kiyomitsu, Marvin van Toorn, Takahiro Yamamoto, Yutei Takahashi, Yumeko Nomura, Ai Kiyomitsu, Toane Arata, and Ayaka Mori.

Abstract

During development of multi-cellular organisms, a fertilized egg undergoes repeated cell division called mitosis. In mitosis, a microtubule-based mitotic spindle is assembled and accurately segregates duplicated chromosomes to daughter cells to maintain genomic information. On the other hand, position and orientation of the mitotic spindle are related to cellular differentiation and tissue morphogenesis during development. Key conserved genes required for mitotic spindle assembly and positioning have been identified in simple model systems. However, their precise mechanisms are still unclear at the molecular and structural level. In additon, considering different features of early embryonic divisions in vertebrates, early embryos may have developed unique mechanisms for spindle assembly and positioning.

In the Cell Division Dynamics Unit, we are studying the mechanisms of mitotic spindle assembly, positioning, and remodeling using cultured human cells and medaka fish embryos to understand the general and context-dependent mechanisms for chromosomal stability and cell fate regulation in vertebrate mitosis.

1. Staff

- Tomomi Kiyomitsu, Assistant Professor (PI)
- Ai Kiyomitsu, Researcher
- Marvin van Toorn, Postdoctoral fellow (JSPS fellow)
- Takahiro Yamamoto, Postdoctoral fellow
- Ayaka Mori, Research Technician
- Toane Arata, Research Technician
- Yang Ming, Graduate Student
- Yutei Takahashi, Rotation Student
- Muhammad Hamzah, Rotation Student
- Yumeko Nomura, Intern Student

- Yoko Nakasone, Research Assistant
- Tomomi Teruya, Research Unit Administrator

2. Collaborations

2.1 Visualization of mitotic spindle assembly in medaka early embryos

- Description: Visualizing dynamics of EGFP-alpha-tubulin in live medaka embryos
- Type of collaboration: Joint research
- Researchers:
    - Professor Minoru Takana, Nagoya University
    - Dr. Toshiya Nishimura, Nagoya University (currently Hokkaido University)

2.2 CRISPR and auxin-inducible degron in medaka embryos

- Description: Applying auxin-inducible degron 2 (AID2) for medaka embryos.
- Type of collaboration: Joint research
- Researchers:
    - Professor Masato Kanemaki, National Institute of Genetics
    - Dr. Satoshi Ansai, Tohoku University (currently Kyoto University)

2.3 Visualizing cytoplasmic streaming in medaka embryos

- Type of collaboration: Joint research
- Researchers:
    - Ms. Luolan Bai, Harvard Medical School
    - Professor Tim Mitchison, Harvard Medical School

2.4 Photo-switchable microtubule inhibitor

- Type of collaboration: Joint research
- Researchers:
    - Professor Akimitsu Narita, OIST
    - Dr. Jingyun Tan, OIST

3. Activities and Findings

3.1 Mechanisms of spindle assembly in medaka early embryos

A gradient of GTP-bound form of Ran (Ran-GTP) has been recognized as one of chromosome-derived signals that facilitate spindle assembly around chromosomes. Prior studies demonstrated that the Ran-GTP gradient is critical for acentrosomal spindle assembly in female meiosis, but dispensable for bipolar spindle formation in somatic human cells (Tsuchiya et al., Current Biology 2021). Although multiple pathways including Ran-GTP and centrosomes coordinately assemble mitotic spindles in somatic cells, it remains unclear how these pathways contribute to organizing large embryonic spindles in vertebrates. In this fiscal year, we analyzed requirement of Ran-GTP for spindle assembly in medaka embryos using dual-color live imaging, a dominant negative Ran mutant, and an auxin inducible degron2 (AID2)-based protein knockdown system. In contrast to typical somatic spindles, we found that embryonic spindles have two unique features: a dense microtubule network around chromosomes and precocious centrosome separation from spindle poles during mitosis. Importantly, depletion of RCC1, a GEF for Ran, diminished the dense microtubule network around chromosomes and caused severe chromosome segregation defects in early embryonic divisions, but not in later blastula stage. Together, we propose that despite the presence of centrosomes, the chromosome-derived Ran-GTP pathway has essential roles in functional spindle assembly in large, rapidly dividing vertebrate early embryos, similar to acentrosomal spindle assembly in oocytes. These results were published in Kiyomitsu et al., Nature Communications on February, 2024.

In parallel, we have also studied the localization and function of multiple Ran's targets and microtubule-binding motors in medaka early embryos. Especially, we succeeded in visualizing and depleting dynein motor in medaka embryos (Kiyomitsu et al., in preparation). Furthermore, we analyzed functional domains of RCC1 using RCC1 mutants, and tested other methods in medaka early embryos.

3.2 Mechanisms of spindle assembly and maintenance in human cells

Cytoplasmic dynein and nuclear mitotic apparatus (NuMA) protein are well-conserved spindle-pole localizing proteins in vertebrates. Accumulating evidence indicates that NuMA targets and activates dynein at the minus-end of spindle microtubules to provide robust clustering of microtubules into a focused, bipolar spindle. However, it remains unclear how dynein and NuMA forms complex specifically during mitosis, and how these macro-molecular complex functions to maintain microtubule minus-end focusing at the spindle poles in human metaphase cells. In this fiscal year, we analyzed cell-cycle dependent modifications of dynein, NuMA and their associated proteins using mass spectrometry. We found key modifications, which are critical for their complex formation. In addition, we succeeded in establishing several auxin-inducible degron (AID)-tag knock-in cell lines using non-transformed Rpe1

cells. We performed AID-mediated protein knockdown and confirmed some results obtained last year using transformed HCT116 cells. We are now preparing a manuscript on how dynein, dynactin and NuMA function for the maintenance of spindle pole focusing during metaphase in human cells.

4. Publications

4.1 Journals

1. Ran-GTP assembles a specialized spindle structure for accurate chromosome segregation in medaka early embryos
   Kiyomitsu A**,** Nishimura T, Hwang SJ, Ansai S, Kanemaki MT, Tanaka M, Kiyomitsu T.
   Nature Communications 2024 Feb 1;15(1):981. doi: 10.1038/s41467-024-45251-w.

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

1. (Oral) Kiyomitsu T. *Ran-GTP promotes zygotic spindle assembly in Medaka Oryzias latipes*, Mitotic Spindle: From living and synthetic systems to theory, Dubrovnik, Croatia, 16-19 April (2023).
2. (Oral) Kiyomitsu T. *Mechanisms of spindle assembly and maintenance in somatic cells and medaka fish embryos*, The 49th Naito Conference, Sapporo, Japan, 4-7 July (2023).
3. (Oral) Kiyomitsu T. *Mechanisms of spindle assembly and maintenance in somatic cells and medaka fish embryos*, Hokkaido University, Sapporo, Japan, 7 July (2023).
4. (Oral) Kiyomitsu T. Ran-GTP assembles a specialized spindle structure for accurate chromosome segregation in medaka early embryos. Cell Division Workshop 2023, Mishima, Japan, 27-28, July (2023)
5. (Oral) Kiyomitsu T. *Mechanisms of spindle assembly in medaka fish embryos*, Cell Bio 23, Boston, USA, 2-6 December (2023)
6. (Poster) van Toorn M. Gooch A, Boerner S, and Kiyomitsu T. NuMA deficiency causes micronuclei via checkpoint-insensitive k-fiber minus-end detachment from mitotic spindle poles, MBSJ 2023, Kobe, Japan, 6-8 December (2023)

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

6.1 Cell Biology Across Boundaries: ASCB-EMBO-MBSJ Joint International Workshop, Subgroup in Cell Bio 23.

- Date: December 3, 2023
- Venue: Boston Convention & Exhibition Center, Boston
- Co-organizers: Iain M. Cheeseman (MIT) & Tomomi Kiyomitsu (OIST)
- Speakers:
    - Dr. Silke Hauf (Virginia Tech, VA)
    - Dr. Samara Reck-Peterson (UCSD, HHMI)
    - Dr. Fumio Motegi (Hokkaido University)
    - Dr. Monica Bettencourt-Dias (Fundação Calouste Gulbenkian)
    - Dr. Amy Gladfelter (Duke University, HHMI)
    - Dr. Tomomi Kiyomitsu (OIST)

6.2 Cell and Molecular Biology seminar:

- Date: June 7, 2023
- Venue: OIST Campus Lab4 F01
- Speaker: Dr. Tomomi Kiyomitsu (OIST)

6.3 OIST BOG meeting:

- Date: September 28, 2023
- Venue: OIST Campus C209
- Speakers:
    - Dr. Makoto Yamada (OIST)
    - Dr. Kazumasa Tanaka (OIST)
    - Dr. Gerald Pao (OIST)
    - Dr. Tomomi Kiyomitsu (OIST)
    - Dr. Philipp Andres Hoehn (OIST)

7. Other

External funding

- Tomomi Kiyomitsu: JST FOREST grant, KAKENHI-B