Science and Technology Group
Annual Report FY2024

Ai Kiyomitsu
Science and Technology Associate

1 Introduction

During cell division, a microtubule-based structure called spindle segregates duplicated chromosomes to daughter cells to maintain genomic information. In animal mitosis, the spindle is assembled by multiple pathways including centrosomes and a chromosome-derived Ran-GTP gradient. 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 cell line with centrosomes (Tsuchiya et al., *Current Biology* 2021). Although spindle assembly mechanisms have been extensively studied in oocytes and somatic cells, mechanisms for centrosomal spindle assembly in large vertebrate embryos remain poorly understood (Figure 1).

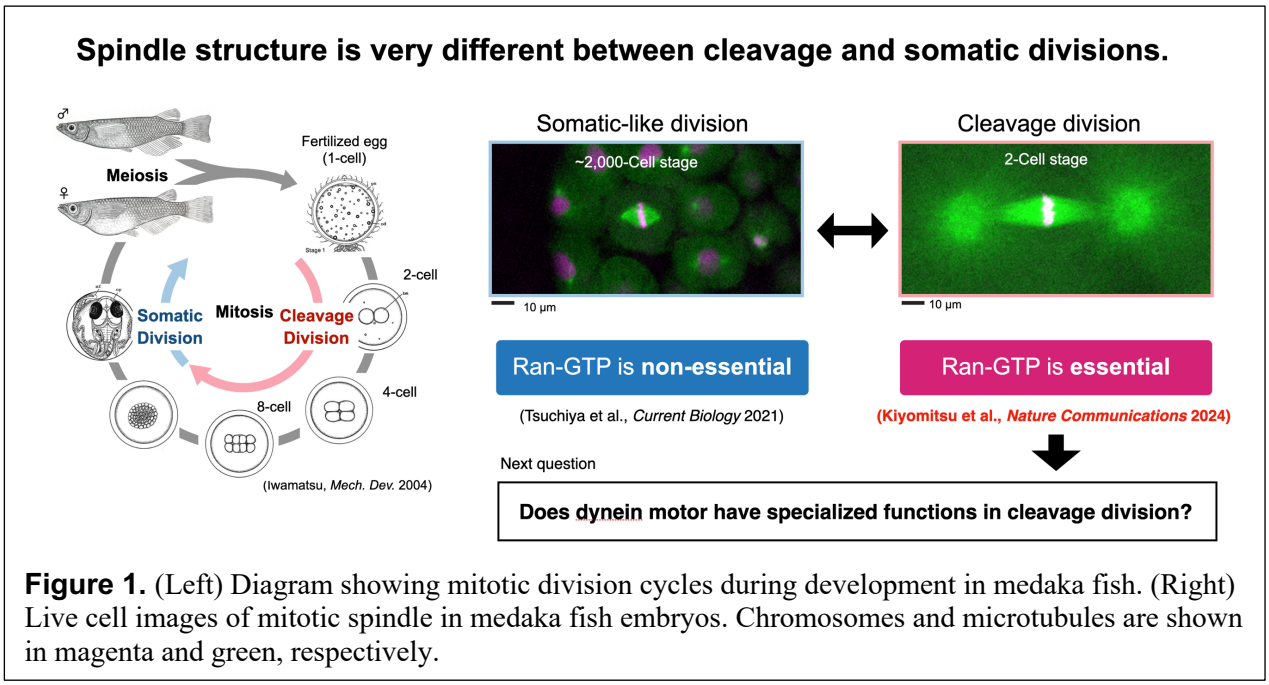

Figure 1. (Left) Diagram showing mitotic division cycles during development in medaka fish. (Right) Live cell images of mitotic spindle in medaka fish embryos. Chromosomes and microtubules are shown in magenta and green, respectively.

To understand the mechanisms of spindle assembly in medaka early embryos, I have established live functional assay systems by combining CRISPR knock-in with live imaging and auxin-inducible degron (AID) technology. We found that Ran-GTP pathway has essential roles in functional spindle assembly in large, rapidly dividing medaka early embryos (Figure 1, Kiyomitsu et al., *Nature Communications* 2024).

2 Activities and Findings

Based on the publication, I applied for NIKON imaging award with Tomomi Kiyomitsu, and received NIKON JOICO AWARD.

To understand spindle assembly mechanisms in medaka early embryos, I have next sought to study microtubule-based motor dynein. Dynein is a conserved minus-end directed motor that has multiple essential roles in mitotic spindle assembly and positioning in somatic cells. However, dynein's localization dynamics and function in extremely large vertebrate embryos remain unclear.

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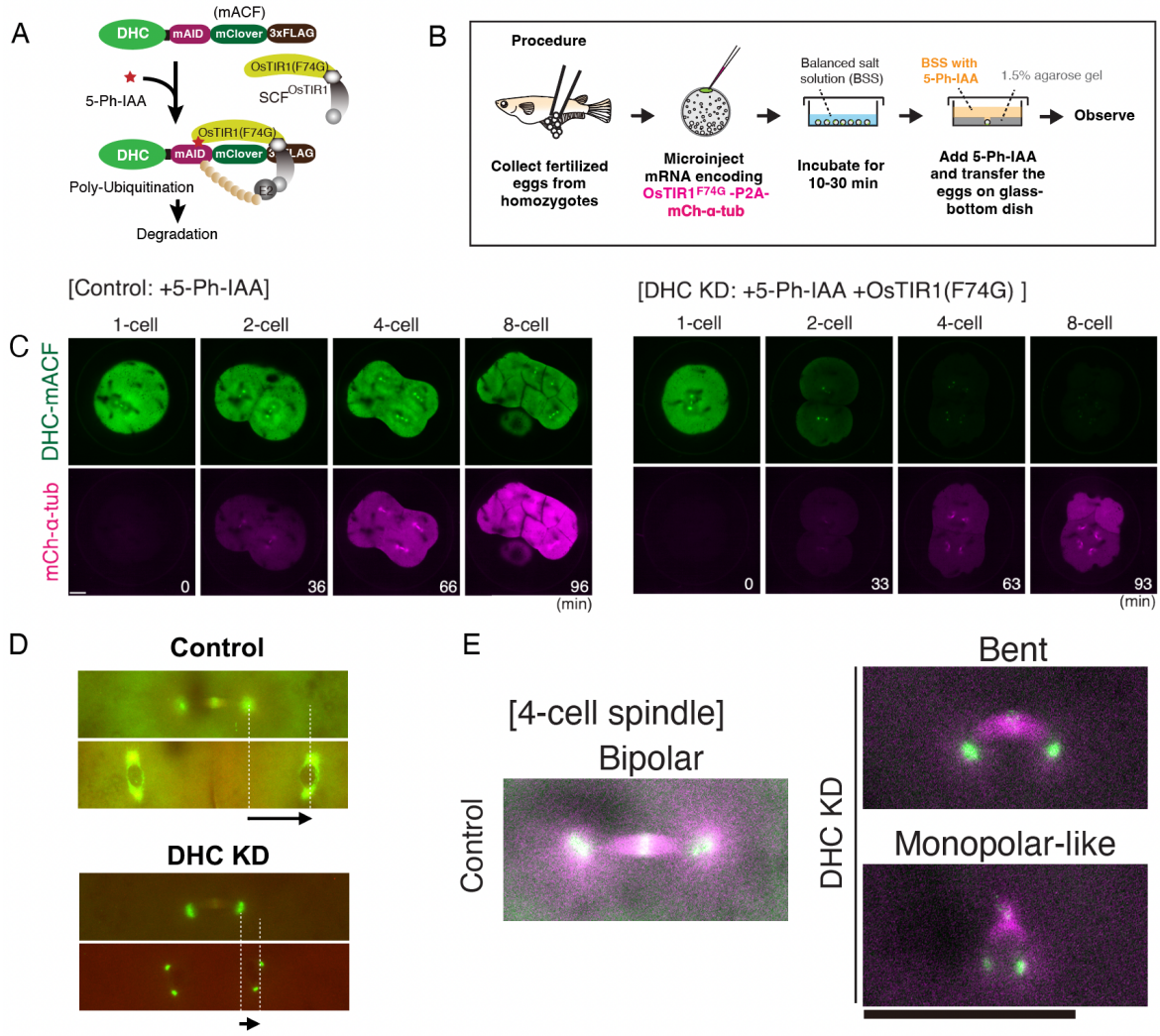

Figure 2. (A) Schematic representation of auxin inducible degron 2 (AID2)-mediated DHC degradation. (B) Procedure of AID-mediated protein knockdown in medaka fertilized eggs. (C) Representative live-cell images showing the fluorescence of DHC-mACF and mCh-alpha-tubulin in control (left) and DHC knockdown (KD, right) embryos. (D-E) Phenotypes in nucleus and centrosome positioning (D) and spindle structure (E) in DHC KD embryos. Scale bars = 100 μm

In FY2024, I have analyzed localization and functions of dynein (dynein heavy chain, DHC) and its binding partner, dynactin (p50 subunit), in early- and late-stage medaka embryos using CRISPR, live cell imaging, and auxin-inducible degron 2 (AID2)-mediated protein knockdown system (Figure 2). I found that both dynein and dynactin localize at multiple subcellular sites including centrosomes, nuclear envelop, kinetochores and spindle microtubules. Interestingly, both dynein and dynactin accumulate at metaphase spindle center and 'halo'-like cytoplasmic region in early embryos. I also found that AID-mediated depletion of dynein or dynactin causes centrosome mis-positioning at NEBD, which leads to abnormal spindle formation followed by chromosome mis-segregation and cytokinesis defects in early embryos. These results were presented in ASCB/Cell Bio 24 international conference.

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3 Collaborations

Kiyomitsu Unit (OIST)
Prof. Minoru Tanaka (Nagoya University)
Dr. Toshiya Nishimura (Hokkaido University)
Dr. Satoshi Ansai (Okayama University)

4 Publications and other output

<Award>

Kiyomitsu A and Kiyomitsu T.
2024 NIKON JOICO AWARD

<Poster presentation>

Kiyomitsu A, Mori A, Kiyomitsu T.
Dynein-mediated centrosome positioning ensures bilateral microtubule-nucleation waves for rapid bipolar spindle assembly in medaka embryos
ASCB/Cell Bio 24, San Diego, Dec 18, 2024