Science and Technology Group
Annual Report FY2023

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.

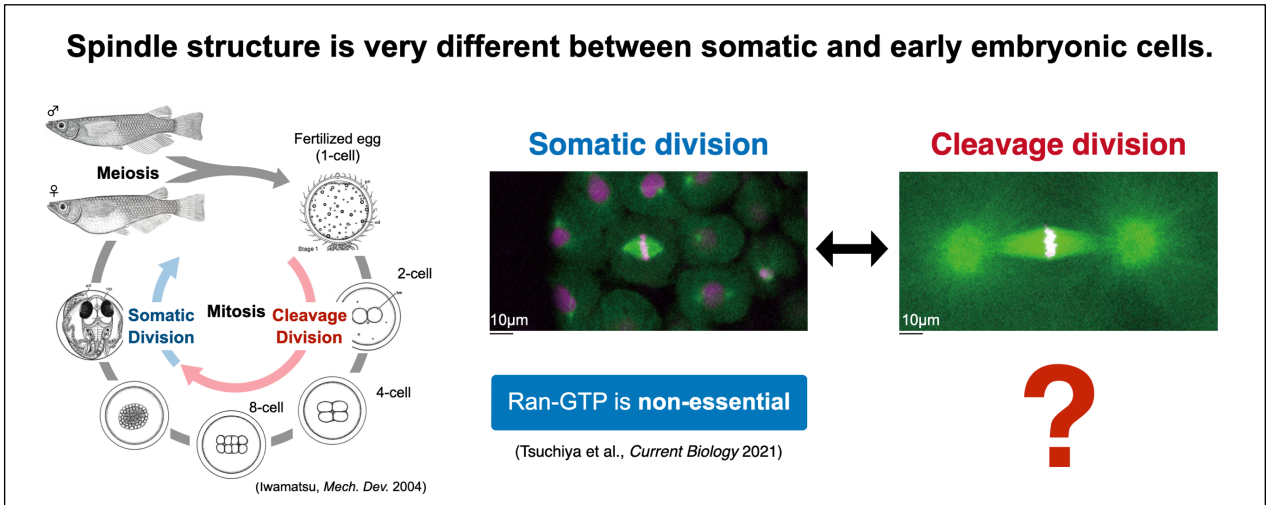

Figure 1. (Left) Diagram showing mitotic division cycles during development in Medaka fish. (Right) Live cell images of mitotic spindle in Medaka fish embryos. Chromosomes and microtubules are shown in Magenta and Green, respectively.

2 Activities and Findings

To understand the mechanisms of spindle assembly in Medaka early embryos, I analyzed requirement of Ran-GTP using CRISPR knock-in, live imaging, a dominant negative Ran mutant, and an auxin inducible degron2 (AID2)-based protein knockdown system. We submitted a research paper in July 2023 and received reviewers' comments in August. To address them, I performed additional experiments including immune-fluorescence (IF) and microtubule regrowth assay after cold-treatment of mitotic spindles. Also, I repeated live-imaging experiments to increase sample numbers and performed image quantifications. We submitted the revised manuscript, which were finally accepted in January 2024 in Nature Communications. Some of my new results are shown in Figure 2, which shows that Ran-GTP plays essential roles in medaka early embryos. Based on this achievement and next questions, I applied for a KAKENHI Kiban-C grant, which was fortunately accepted.

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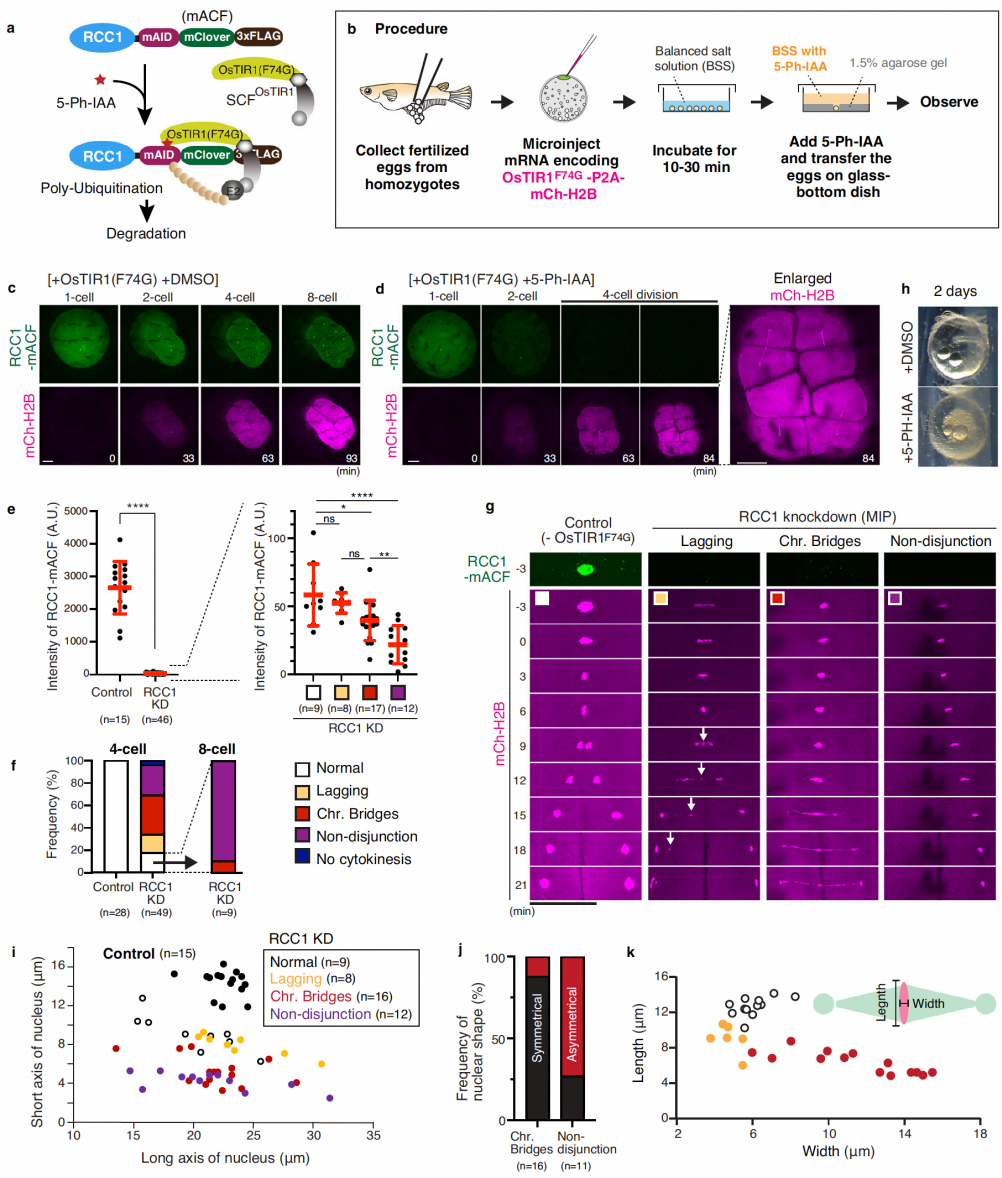

Figure 2 (adapted from Kiyomitsu et al., Nat Commun. 2024) AID2-mediated degradation of endogenous RCC1 causes severe chromosome segregation defects in medaka early embryos. For details, please see Figure 7 of Kiyomitsu et al., Nat Commun 2024.

3 Collaborations

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

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4 Publications and other output

<Publication>

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

<Grant acquisition>

KAKENHI Kiban-C from 2024-04-01 to 2027-03-31