Science and Technology Group Annual Report FY2022

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.

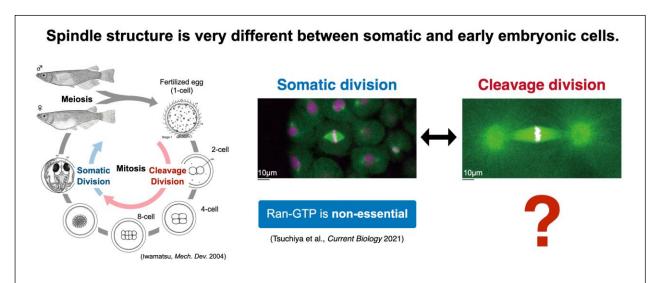


Figure 1. (Left) Diagram showing mitotic division cycles during development in Medaka fish. (Right) Live cell images of mitotic spindle in Medaka fish embryos. Chromosomes and microtubules are shown in Magenta and Green, respectively.

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. Most importantly, I could establish AID2 system (Figure 2A) in Medaka embryos in this fiscal year. Using this technique, I found that depletion of RCC1, a guanine-nucleotide exchange factor for Ran, diminishes the microtubule-dense region around chromosomes in zygotic spindles (Figure 2A), which results in abnormal chromosome segregation including anaphase lagging, chromosome bridge, and chromosome non-disjunction (Figure 2B). Based on these and other results, we are currently preparing a manuscript.

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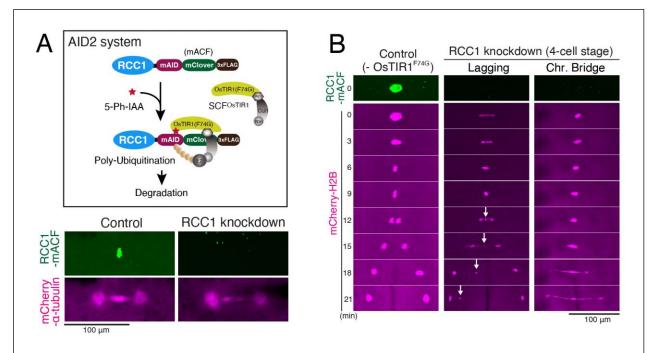


Figure 2. (A) (Top) Diagram showing auxin-inducible degron 2 (AID2) system. (bottom) Depletion of RCC1-mAID-mClover-FLAG (mACF) by AID2-mediated protein knockdown. (B) Live cell images of chromosomes in control (left) and RCC1 depleted Medaka embryos (right). Chromosomes shown in Magenta are abnormally segregated in RCC1-depleted cells (Ai Kiyomitsu et al., *in preparation*).

3 Collaborations

Kiyomitsu Unit (OIST)

Prof. Minoru Tanaka (Nagoya University)

Dr. Toshiya Nishimura (Hokkaido University)

Dr. Satoshi Ansai (Tohoku University)

4 Publications and other output

<Poster presentation>

Ai Kiyomitsu, Ran-GTP promotes zygotic spindle assembly for accurate chromosome segregation in Medaka Oryzias latipes. OIST STG forum, March 28, 2023.

<Grant acquisition>

SHINKA grant, Representative: Tomomi Kiyomitsu, Collaborator: Ai Kiyomitsu, Satoshi Ansai (Tohoku University)

JST FOREST grant (7 years from 2023-2029), Representative: Tomomi Kiyomitsu, Collaborator: Ai Kiyomitsu