Science and Technology Group
Annual Report FY2022

Midori Ota
Science and Technology Associate

Mechanisms of Centrosome Assembly and Activation in cell division

1 Introduction

My work focuses on centrosomes, small organelles that are the major microtubule organizing centers in animal cells. During cell division, centrosomes catalyze the formation of the mitotic spindle that segregates the replicated chromosomes into the daughter cells (**Fig. 1**). Centrosomes are composed of a centriolar core that organizes a proteinaceous matrix called pericentriolar material (PCM) that docks γ-tubulin containing complexes (γTuCs) to nucleate microtubules. Centrosome amplification causes aneuploidy and drives tumor formation, while defects in centrosome assembly and activation can lead to neurodevelopmental disorders such as microcephaly. Understanding the molecular mechanisms of centrosome assembly and activation is crucial to identify possible targets and disease-specific therapeutic approaches.

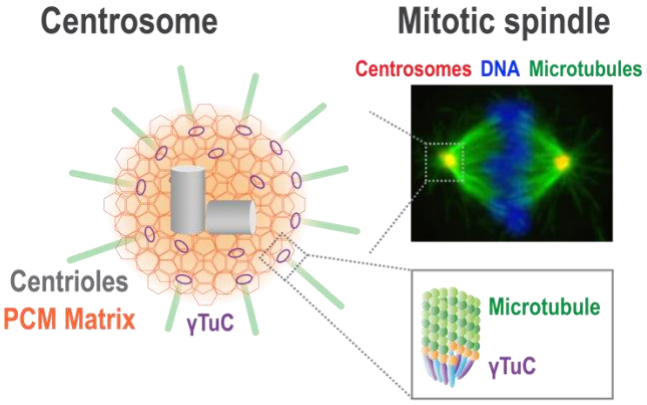

Figure 1. Centrosomes catalyze mitotic spindle assembly for chromosome segregation.

2 Activities and Findings

During mitotic entry, the PCM matrix that surrounds the centrioles increases in size and microtubule nucleating capacity in a process called centrosome maturation (**Fig. 2**). Polo-like kinase 1 (PLK1) is recruited to the centrosome and phosphorylates PCM matrix proteins, which leads to PCM expansion. By combining biochemical reconstitution and live-cell imaging in *Caenorhabditis elegans* embryos, we revealed that in addition to controlling PCM expansion, PLK1 independently controls the generation of binding sites for γTuCs on the PCM matrix by phosphorylating two distinct regions of the PCM matrix protein SPD-5 (homolog of human CDK5RAP2 and Drosophila Cnn) (**Fig. 2**). Selective inhibition of the PLK1-dependent γTuC docking sites in SPD-5 led to spindle defects and impaired chromosome segregation without affecting PCM expansion, highlighting the importance of phospho-regulated centrosomal γTuC docking sites in spindle assembly (**Ohta et al, JCB, 2021**). This finding has raised the question of ***how phosphorylation of SPD-5 generates γTuC docking sites and activates microtubule nucleation.***

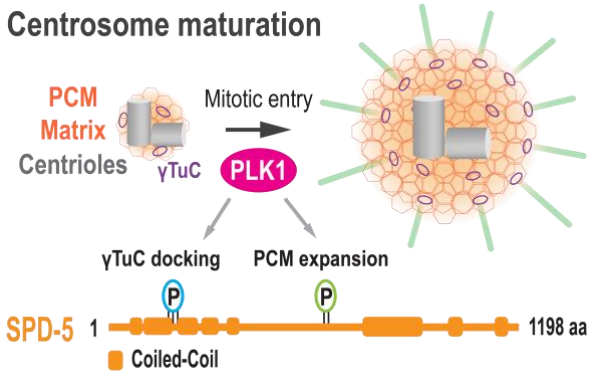

Figure 2. PLK1 transforms the centrosome in preparation for mitosis, leading to an increase in PCM matrix size and allowing the docking and activation of γTuCs.

In FY22, we found that the PLK1 phosphorylation converts the *C. elegans* matrix molecule SPD-5 into a mitotically active γTuC docking site (**Fig. 3**). We identified two γTuC binding domains in the SPD-5 N-terminus that exhibit PLK1 phosphorylation-dependent γTuC binding. Our results from the biochemical reconstitution assay suggested an autoinhibitory interaction between these two γTuC binding sites. We also revealed that PLK1 phosphorylation increases the hydrodynamic radius of the

SPD-5 N-terminus without altering its native molecular weight, indicative of a conformational change. Collectively, these results suggest that phosphorylation by PLK1 triggers a reorganization of the SPD-5 N-terminus that generates an extended interaction surface consisting of two distinct γTuC binding sites. Selective activation of γTuC docking on PCM matrix molecules by PLK1 phosphorylation likely couples PCM expansion to the increased centrosomal microtubule nucleation that drives efficient spindle formation.

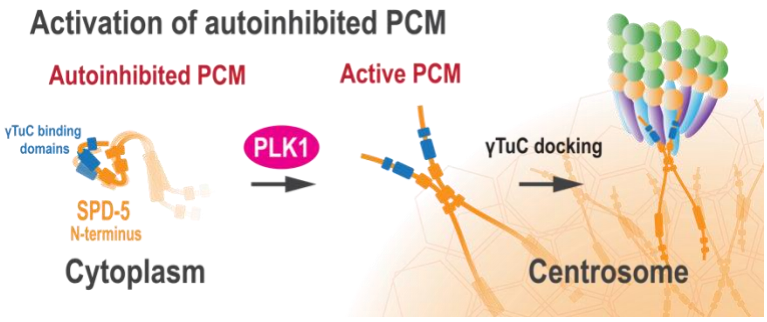

Figure 3. The SPD-5 N-terminus undergoes a conformational change upon PLK1 phosphorylation.

3 Collaborations

External collaborations:

1) Karen Oegema and Arshad Desai (University of California, San Diego)
2) Yajie Gu and Kevin Corbett (University of California, San Diego)
3) Jeffrey Woodruff (UT Southwestern Medical Center)

Internal collaborations:

1) Orie Arakawa and Franz Meitinger (OIST, Cell Proliferation and Gene Editing Unit)

4 Publications and other output

Publications

1) Jack Houston, **Midori Ohta**, J. Sebastián Gómez-Cavazos, Amar Deep, Kevin D. Corbett, Karen Oegema, Pablo Lara-Gonzalez, Taekyung Kim, Arshad Desai. PLK-1 tethered on BUB-1 directs CDC-20 kinetochore recruitment to ensure timely embryonic mitoses. bioRxiv 2022.10.07.511323; doi: https://doi.org/10.1101/2022.10.07.511323

Conferences: Invited talks

1) Workshop: Protein assemblies driving cell division, The 45th Annual Meeting of the Molecular Biology Society of Japan, Makuhari, Japan, November 30-December 2, 2022.
2) Cold Spring Harbor Asia meeting, Cilia & Centrosome, Awaji, Japan, February 28-March 3, 2023

Conferences: Panelists

1) MBSJ-ASCB-EMBO joint workshop Part 2: Navigating your career across boundaries, The 45th Annual Meeting of the Molecular Biology Society of Japan, Makuhari, Japan, November 30-December 2, 2023.