別紙 4

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主　論　文　の　要　旨

論文題目 Identification and analysis of mechanisms that bypass the essentiality of Polo, a mitotic regulator
(有糸分裂制御因子 Polo の必須性をバイパスする機構の同定と解析)

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論　文　内　容　の　要　旨

Mitosis is a fundamental cellular process in which chromosomes are accurately segregated into two daughter cells. This process requires the assembly of dynamic filaments and microtubules (MTs), and is collectively regulated by various kinases and phosphatases. The processes involved in mitosis are generally well-conserved among eukaryotes. Paradoxically, the set of mitotically essential genes varies among different species. This indicates that the cellular functions of mitotically essential genes can be replaced or bypassed by alternative pathways during evolution. However, despite extensive efforts, bypass of essentiality (BOE) events for mitotic genes have rarely been reproduced in laboratory experiments. Therefore, many open questions remain regarding the BOE of mitotic regulators (BOE-M). For example, which “essential” genes can be substituted by other genes and become non-essential? What kinds of cellular functions and pathways function in the absence of mitotically essential genes? How can cells achieve BOE-M under natural conditions without artificial mutagenesis?

To uncover a novel BOE-M, extragenetic suppressor screening for mitotically essential gene disruptants was conducted using mutagenesis and subsequent evolutionary repair experiments in the fission yeast, *Schizosaccharomyces pombe*. Viable cells were isolated when the coding region of polo-like kinase (Plk) was completely removed. This was unexpected because Plk is a versatile and essential mitotic kinase in many cell types. Whole genome sequencing of

viable Plk-deleted cells and subsequent experiments on genetic interactions revealed that the BOE of Plk is achieved via 16 suppressor mutations. Gene classification based on the cellular functions of suppressor mutations revealed that the BOE of Plk is enabled by specific mutations in the MT-nucleating γ-tubulin complex, which is expected to be the downstream machinery of Plk, and unexpectedly, through mutations in glucose uptake genes and the protein kinase A (PKA) pathway, which are not readily associated with mitosis. Furthermore, additional genetic screening revealed that the latter bypass was dependent on casein kinase 1 (CK1), which is involved in a delay in the septation initiation network (SIN), but is not considered a major mitotic regulator in *S. pombe*. Our genetic and phenotypic data suggest that CK1 and other suppressor mutations constitute an alternative mechanism of MT nucleation that is normally dominated by Plk. Plk and Ck1 inhibitors caused synthetically skewed bipolar spindle formation and mitotic arrest in a human colon cancer cell line. Therefore, the genetic interaction between Plk and CK1 is conserved in human cell lines.

Our study uncovered a new case of BOE-M in *Plk*, which is an essential mitotic gene to control the cell cycle, nucleate mitotic microtubules at centrosomes, and induce septation initiation. The viability of Plk-deleted cells depended on the recovery of MT nucleation via BOE-M; however, some other phenotypes were not recovered in the viable strains. Thus, BOE analysis enabled the separation of the essential and non-essential cellular functions of Plk in *S. pombe*. From a broad perspective, this study found that BOE-M can be achieved by simple genetic or environmental changes, such as lowering glucose concentration, which can be made even in a natural environment. In addition, unusual aspects of BOE-M, such as the reinforcement of downstream machinery and changes in nutrient pathways, have been revealed that are functionally unrelated. This suggests that BOE-M constitutes a powerful means to uncover a hitherto under-studied mechanism driving mitosis and to understand various aspects of genetic interactions.