Abstract of Thesis

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Title: Possible mechanisms of calcium ions during mitosis
(分裂期におけるカルシウムイオンの機能解析)

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Chapter 1: General introduction

Inefficient transmission of genetic information from a mother cell to daughter cells leads to genetic instability which is commonly found in several cancerous cells. To facilitate the passing of genetic information, the mother cell shortens the long genetic encoding DNA molecule into a compact chromosome structure through a process called chromosome condensation. During mitosis, chromosome dynamics including condensation, alignment and segregation are important for determining the faithful cell division. Inside the cells, Ca^{2+} as a secondary messenger, is involved in many signaling cascades regulating cell cycle progression. Increasing of Ca^{2+} concentration was detected during mitosis (Whitaker, 2006). Previous reports have revealed that divalent cations (Mg^{2+}, Ca^{2+}) exhibit a localization shift from cytoplasm to chromatin during interphase and mitotic phase (Strick *et al.*, 2001). These suggest a correlation of Ca^{2+} in mitosis, especially with chromosome condensation.

In this study, I aimed to clarify the precise role of Ca^{2+} in two processes important for the faithful mitotic progression: chromosome condensation and kinetochore-microtubule attachment, with the depletion of Ca^{2+} during mitosis. Contribution of Ca^{2+} to chromosome condensation was studied in living cells and isolated chromosomes. Moreover, the contribution of Ca^{2+} to the stability of spindle microtubule-kinetochore attachment which is also important for mitotic progression was also clarified.

Chapter 2: Contribution of calcium ions in chromosome condensation

Several previous studies have revealed the roles of Ca^{2+} during mitosis (Izant, 1983; Hepler, 1994; Keith 1987). However, most of these studies mainly focus on metaphase/anaphase transition and used Ca^{2+}-addition in the investigations. In this study, instead of Ca^{2+}-addition, defects in mitosis when Ca^{2+} was depleted, were observed. Ca^{2+}-depletion prolonged prometaphase stage and increased the frequency of cells with chromosome misalignment. Because chromosome condensation continuously occurs from prophase to prometaphase, depletion of Ca^{2+} which was abundantly found on chromosomes (Levi-Setti *et al.*, 2006), might affect the condensation process, resulting in prolonged prometaphase. Chromosome compaction was quantified in living cells using fluorescence lifetime imaging microscopy-Förster resonance energy transfer (FLIM-FRET) analysis (Llere et al, 2009) and visualized at high resolution in chromosome spreads using scanning electron microscope (SEM). By using HeLa cells expressing H2B-EGFP and mCherry-H2B ($HeLa^{H2B\text{-}2FP}$), chromosome compaction could be quantified by measuring fluorescence lifetime of H2B-EGFP. Chromosome condensation brings the two FP-tagged H2B to be close enough for the energy transfer from excited EGFP to mCherry, resulting in a reduction of fluorescence lifetime of EGFP. After nuclear envelope breakdown, Ca^{2+}-depleted cells had less compacted chromosomes compared with normal cells. The chromosome compaction in the cell could be manipulated by depletion/re-addition of by Ca^{2+}. Using SEM, expanded chromosomes with fibrous chromatin structures were observed in

chromosome spread prepared from Ca^{2+}-depleted cells. Taken together with SEM results, chromosome compaction/expansion induced by the presence/depletion of Ca^{2+} involves a transition of chromatin between fibrous and compact globular structures within chromosomes.

Chapter 3: Contribution of calcium ions in kinetochore-microtubule attachment

Spindle microtubules capture a chromosome through a complex protein network called kinetochore. Chromosome decondensation induced by Ca^{2+}-depletion might affect the formation of kinetochore located at chromosome centromere, compromising the stability of kinetochore-microtubule attachment, in consequence, increasing cells with chromosome misalignment. Kinetochore components are recruited to centromeric chromatin defined by the presence of trimethylated histone H3 lysine 9 (H3K9me3) in stepwise manner from innermost to outermost layers (Cheeseman and Desai, 2008; Przewloka and Glover, 2009). Therefore the localization of several centromeric and kinetochore proteins to centromere during Ca^{2+}-depletion was evaluated. The localizations of centromeric proteins H3K9me3, heterochromatin protein 1 (HP1) remained unchanged even in Ca^{2+}-depleted cells. Moreover, Aurora B kinase, Mis12 and Hec1, anchored kinetochore proteins, were effectively recruited to centromere in Ca^{2+}-depletion. However, CENP-F, a dynamic kinetochore component, was absent from kinetochore-microtubule interface under Ca^{2+}-depletion. This caused the abolishment of cold-stable kinetochore microtubules, implying unstable microtubule-kinetochore attachment. Disruption of CENP-F locating outermost kinetochore layer, but not other interior kinetochore component suggests that loss of CENP-F is unrelated to chromosome decondensation induced by Ca^{2+}-depletion. It means a new Ca^{2+} functions in promoting the stability of kinetochore-microtubule attachment through the regulation of CENP-F recruitment to kinetochore-microtubule interface.

Chapter 4: General conclusion

In this study, dual functions of Ca^{2+} during early stage of mitosis were identified. Increment of intracellular Ca^{2+} during mitosis is important for mitotic progression through promoting chromosome condensation and the stability of kinetochore-microtubule attachment. Ca^{2+} is required for mitotic chromosome compaction by influencing the transition of chromatin from a fibrous structure to a compact globular structure. Moreover, recruitment of dynamic kinetochore components to centromere is also regulated by Ca^{2+}. Taken together, this study uncovers new functions of Ca^{2+} in in mitosis which could be potentially utilized for controlling cells with proliferative defects, such as cancer.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文では細胞分裂の初期におけるカルシウムの役割を明らかにすることを目的とした一連の研究をまとめたものであり、得られた主な成果を要約すると以下のとおりである。

（1）細胞分裂時に細胞内のカルシウムを欠乏させることにより、分裂前中期の時間が延長し、染色体の不整合の頻度が上昇することを見出した。さらにヒストンタンパク質をそれぞれ緑色蛍光タンパク質と赤色蛍光タンパク質と融合させたキメラヒストンタンパク質を用いて、蛍光共鳴エネルギー移動により染色体の凝集状態を解析したところ、カルシウムを除去した場合には、核膜消失後の染色体構造がゆるんでいることを明らかにした。

（2）細胞分裂時に重要な機能を果たしている動原体の構築におけるカルシウムの寄与を明らかにした。その結果、動原体を構成する H3K9Me3、および heterochromatin protein 1 には変化がなく、AuroraB、Mis12、Hec1 なども正常に機能していることを示した一方で、CENP-F が欠失していることを見出した。またこれは動原体と微小管の接着を不安定化させていることを見出した。

以上のように本論文は、従来はカルシウムの添加を中心に解析が進められてきた細胞分裂時のカルシウムの機能解析に対して、細胞内のカルシウムを除去するという新たなアプローチによって、カルシウムが細胞分裂時の染色体の構造の維持と分裂において重要な機能を担っていることを示すものである。よって本論文は博士論文として価値あるものと認める。