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Mitosis is the process by which the sister chromatids are segregated into two daughter cells during eukaryotic cell division. During the mitotic phase of the cell cycle, the intracellular organization is rearranged dramatically to facilitate the assembly of mitosis-specific molecular machineries, such as the mitotic spindle. The mitotic spindle mainly consists of a dense array of microtubules, which provide a framework for the spindle and pull the sister chromatids apart towards the poles of the spindle. Although mitosis is a process common to all eukaryotic cells, different cells adopt different forms of mitosis. The similarities and differences between the different forms of mitosis are important for understanding the evolution and mechanics of mitosis. Open and closed mitosis—two representative types of mitosis—have been well characterized. The two classes are discriminated by whether loss of nuclear compartmentalization (described as nuclear envelope breakdown, or NEBD) occurs or not. Open mitosis involves NEBD. Loss of nuclear compartmentalization promotes spindle formation by allowing that cytoplasmic molecules such as soluble tubulin—a component of the microtubules—gain access to the chromosomes. Moreover, spindle assembly factors (SAFs) are activated locally near the chromosomes through a diffusion gradient of the GTP-bound form of a GTPase, Ran (Ran-GTP). In contrast, closed mitosis occurs in the absence of NEBD. In this process, SAFs are imported into the nucleus by a phase-specific nuclear transport mechanism that is also dependent on Ran. Interestingly, some organisms undergo a type of mitosis that exhibits features of both open and closed mitosis, and is thus categorized as "semi-open" (or "semi-closed") mitosis. For instance, the permeability of the nuclear envelope increases during the prometaphase (as in open mitosis), but the membranous structure remains (as in closed mitosis). Semi-open mitosis is observed in several fungi and invertebrates. However, little is known about this intermediate type of mitosis. How is the intracellular organization rearranged during semi-open mitosis? How does semi-open mitosis contribute to spindle formation? What is the role of the remaining nuclear envelope after its permeability rises? Answering these questions should forward our understanding of the similarities and differences between the different forms of mitosis.

In this study, I investigated the process of semi-open mitosis in *Caenorhabditis elegans* embryos. In *C. elegans*, the permeability barrier of the nuclear envelope, which separates the nucleus from the cytoplasm, is lost during the prometaphase (as in the NEBD of open mitosis). At this point, nuclear proteins such as free histone diffuse into the cytoplasm. This process is commonly known in the *C. elegans* field as NEBD. Here, I refer to this process as "CeNEBD." After the CeNEBD, the nuclear envelope-like structure remains, as confirmed by

optical and electron microscopy. In this thesis, I refer to this structure as "post-nuclear envelope." *C. elegans* semi-open mitosis uses molecules that are also required for spindle formation in open and closed mitosis. Ran is one such molecule. Interestingly, the mechanism by which Ran contributes to spindle formation is different in open and closed mitosis. In open mitosis, Ran-GTP forms a diffusion gradient around the chromosome to stabilize the spindle microtubules, whereas in closed mitosis, Ran-GTP forms a gradient across the nuclear envelope to import factors associated with the microtubules selectively into the nucleus. In the *C. elegans* embryo, knockdown of Ran impairs spindle formation. However, it is still unclear how Ran functions in the *C. elegans* embryo as well as in other organisms that adopt semi-open mitosis. In this thesis, I focused on the intracellular localization of tubulin during semi-open mitosis in the *C. elegans* embryo. Tubulin is the subunit of microtubules and thus a major component of the spindle. I investigated the dynamics of tubulin by using several microscopic techniques and quantitative analyses.

First, I observed the behavior of tubulin during the first cell cycle of the *C. elegans* embryo. I noticed that, prior to the appearance of the filamentous spindle microtubules, uniform signals of tubulin accumulate in the nascent spindle region at the onset of spindle formation. I hypothesized that free tubulin accumulates in the nascent spindle region prior to spindle formation. To confirm this hypothesis, I observed unpolymerized tubulin by disrupting tubulin polymerization. I found that unpolymerized tubulin accumulates in the nuclear region after CeNEBD. I found that several molecules involved in spindle formation also accumulate in the nuclear region. The accumulation of free tubulin and other proteins in the nascent spindle region may facilitate spindle formation during semi-open mitosis.

Second, I determined the timing at which tubulin starts accumulating. I monitored and quantified the entry kinetics of tubulin into the nucleus in parallel with the timing of CeNEBD. This analysis revealed that tubulin accumulation is initiated at the same time as CeNEBD. Importantly, in nocodazole-treated embryos, the timing of CeNEBD differs between male and female pronuclei. The accumulation timing of tubulin also differed between male and female pronuclei, and coincided with the CeNEBD of each pronucleus. This result suggests that CeNEBD triggers tubulin accumulation. It has been reported that nuclear pore proteins disassemble around the CeNEBD. Therefore, I concluded that the selective transport across the nuclear membrane is not responsible for the accumulation of tubulin subunits.

Third, to obtain molecular insights into the mechanism of tubulin accumulation, I investigated whether Ran (*ran-1*) is required for tubulin accumulation in the *C. elegans* embryo. In *ran-1*/Ran-knockdown embryos, tubulin accumulation in the post-nuclear region was

impaired. *ran-1*/Ran is thus required for tubulin accumulation in the post-nuclear region. As reported previously, *ran-1*/Ran-knockdown embryos failed to form the spindle. Tubulin accumulation correlates with successful spindle formation and might thus be important for spindle formation. In open mitosis, Ran-GTP is distributed according to a diffusive concentration gradient originated from the chromosomes. SAFs are activated by Ran, and thus spindle formation is activated around the chromosomes. To test if the Ran-GTP gradient from the chromosomes also defines the region of tubulin accumulation in *C. elegans*, I examined the shape of the region of tubulin accumulation. The shape of the region deformed after CeNEBD, and is thus unlikely defined by a simple diffusion gradient from the chromosomes. Instead, the boundary of the region of accumulation coincided with the post-nuclear envelope after CeNEBD. The observations implied 3 features of the accumulation of tubulin during semi-open mitosis in the *C. elegans* embryo. First, the definition of the specific intracellular region requires Ran, but in a different manner from the definition in open (Ran-GTP diffusion gradient) or closed mitosis (nuclear pore complex [NPC]-dependent transport across the nuclear membrane). Second, the post-nuclear membrane may define the boundary of the region of tubulin accumulation. Third, the spindle may provide a backbone for shaping the post-nuclear membrane, because the membranous structure is deformed when spindle formation is impaired by nocodazole treatment.

How does tubulin accumulate in the post-nuclear region? I examined the dynamics of the accumulated tubulin inside the post-nuclear region by FRAP (fluorescence recovery after photobleaching) analysis. The FRAP analysis revealed that the accumulation is accomplished by a dynamic equilibrium between the post-nuclear region and the cytoplasm. I next quantified the half-life time of the fluorescence recovery and the rate of the immobile fraction. Both parameters did not show significant differences between the post-nuclear region and the cytoplasm. The observation contradicted the stable binding of tubulin to a less mobile structure inside the post-nuclear region. To quantitatively evaluate possible models and parameters that explain the obtained experimental results, I constructed a numerical model that simulates the accumulation of tubulin and the FRAP. The diffusion coefficient of cytoplasmic tubulin estimated from the comparison between computer simulation and experiments was consistent with previously published results and with the RICS (raster image correlation spectroscopy) analysis performed in the present study. The transport rate across the post-nuclear envelope was estimated to be comparable to the diffusion to explain the fast accumulation of the tubulin. I propose a numerical model that explains tubulin accumulation and the FRAP kinetics. In this model, tubulin diffuses freely across the post-nuclear envelope after CeNEBD and is trapped

transiently to a hypothetical immobile substrate inside the post-nuclear region.

In this study, I found that tubulin and other components involved in spindle formation accumulate inside the post-nuclear envelope during semi-open mitosis in the *C. elegans* embryo. This finding indicates a novel mode of rearranging intracellular compartments during mitosis. Collectively, my findings provide new insights in the understanding of the mechanism and function of semi-open mitosis.

博士論文の審査結果の要旨

生命が誕生して約40億年、生物は自らの子孫を増やすために、ひたすら分裂を繰り返してきた。このため、「分裂」は生物にとって、最重要プロセスと言える。ゲノムＤＮＡを収める「核」を獲得し、より複雑な細胞制御が可能となった真核生物は、大きく分けて２つの分裂様式を持つことが知られている。分裂時、核が２つにくびれて分裂が起こる closed mitosis と、核が分裂前に崩壊し、染色体がむきだしになって分裂がおこる open mitosis である。しかし、近年、この２つの様式の中間に位置する semi-open mitosis の存在が知られるようになってきた。open mitosis と closed mitosis は、単に分裂時の核膜の有無だけでなく、染色体分配装置である紡錘体形成と、その制御において大きな違いが存在するため、semi-open mitosis の解析は細胞分裂のメカニズムや、分裂様式の進化の理解において非常に重要である。

林さんは線虫（*C. elegans*)の初期胚において、この semi-open mitosis の研究をおこない、次に述べる４つの興味深い知見を得た。１．分裂時、透過性の増した核（post-nucleus と名付けられた）にチューブリンや他の分裂期関連分子の顕著な蓄積を見出した。２．チューブリンの蓄積は、核膜の透過性が上昇するのに伴って開始された。３．チューブリンの蓄積と紡錘体形成は低分子量 G 蛋白質である RanGTPase が必要であった。４．チューブリンが蓄積されている領域の境界は、post-nucleus の核膜と一致していた。

染色体分配装置である紡錘体は、チューブリンが重合した微小管が束ねられたものである。このため、紡錘体を形成させる領域で、特異的に微小管を重合させる必要があると思われるが、試験管内の実験ではチューブリンはある閾値濃度以上でないと重合を開始しない。これまで、染色体周りの Ran GTPase の活性勾配を利用して微小管の重合を促進する因子群(SAFs)を活性化する機構が知られていた。また、細胞分裂時に核膜が崩壊しない closed mitosis を持つ酵母では、分裂時特異的に微小管の材料であるチューブリンを核内に選択的に輸送する機構が存在する。

林さんは、先に述べた４つの知見のように、semi-open mitosis をもつ線虫の初期胚細胞で、チューブリンが post-nucleus の微小管形成領域に濃縮する機構があることを見い出した。そして林さんは、この濃縮が微小管重合、紡錘体形成促進に重要であると提唱した。これは紡錘体を細胞内の特定の領域で形成させるための新規のメカニズムである。また、post-nucleus の濃縮領域の規定は、RanGTPase を必要とするが、単純な活性濃度勾配でも膜内外の選択的輸送でもなさそうなことから、新規の RanGTPase の役割の可能性も示唆している。

本研究は、今まであまり解析がおこなわれてこなかった semi-open mitosis に焦点を当て、微小管重合が盛んに起こっている post-nucleus 領域でのチューブリンの濃縮機構を明らかにした。FRAP (Fluorescence recovery after photobleaching)などでチューブリンの拡散係数を測定し、数理モデルによる濃縮機構についての考察も加えている。また、通常の経時

観察では発見が困難と思われるチューブリンの濃縮を微小管重合阻害剤を巧みに使って見出したことは特筆すべきことである。得られた結果は、細胞分裂期における微小管重合、紡錘体形成促進の理解に新しい知見を与えるだろう。細胞分裂のメカニズムだけでなく、細胞分裂機構の進化という点でも非常に興味深い。よって林華子さんの学位提出論文は、博士号授与の要件を満たすと審査員全員一致で判断した。