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学位論文題目　Discovery of asymmetric localization of the low-density region in the one-cell stage egg of *Caenorhabditis elegans* and elucidation of its mechanism

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博士論文の要旨

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論文題目：Discovery of asymmetric localization of the low-density region in the one-cell stage egg of *Caenorhabditis elegans* and elucidation of its mechanism

The formation of cell polarity is an important event in cell biology. Cell polarity is critical for cell motility, the determination of the body axis, and asymmetric division. *Caenorhabditis elegans* is a useful model organism to study cell polarity. After fertilization, the one-cell stage egg of *C. elegans* establishes a cell polarity corresponding to the anterior-posterior axis. The cell polarity is associated with the asymmetric localization of PAR proteins, which are conserved molecules across species regulating cell polarity. While the asymmetry in the molecular composition inside the egg was well understood, little was known about the asymmetry in physical properties, such as the distribution of mass density.

A preliminary observation in our laboratory, conducted before I started this study, indicated that the one-cell stage egg of *C. elegans* was aligned in a specific direction against a centrifugal force. I got interested in this phenomenon because it suggested a non-uniform distribution of the mass density in the polarized egg. I found that the region between the eggshell and the cell, which is called the extra embryonic matrix (EEM), had a lower mass density compared to the other part of the egg. The

EEM was larger on the anterior side. This meant the P0 cell of the one-cell stage egg was asymmetrically localized toward the posterior inside the eggshell. My time-lapse observation revealed that the P0 cell shifted toward the posterior side by the timing of the relaxation of the pseudo-cleavage furrow. Furthermore, I observed the P0 cell after removing the eggshell. This analysis revealed the P0 cell apparently migrated toward the posterior side during the relaxation of pseudo-cleavage furrow and also during the asymmetric cell division. Based on my gene knockdown experiments, I propose that the migration of the P0 cell toward the posterior side is driven by the constriction and relaxation of the cell cortex at the lateral sides of the moving direction, and the asymmetry of the contractility of the cell cortex along the moving direction.

This thesis contains three chapters. In Chapter 1, I focused on the phenomenon of the alignment of the long axis of the one-cell stage egg of *C. elegans* along the direction of centrifugal force when I observed the embryo using a centrifuge polarizing microscope (CPM). The phenomenon suggested that the anterior side of the egg had a lower mass density. I analyzed optical path difference (OPD) maps of the egg obtained using an orientation independent differential interference contrast (OI-DIC) microscope. I discovered that the EEM had very low density. I also found that the EEM was localized at the anterior side. The results collectively indicated that the EEM asymmetry was responsible for the asymmetry in the mass density of the egg.

In Chapter 2, I investigated the temporal change of the EEM localization to

determine when the EEM asymmetry was established. I found that the EEM asymmetry was established by the relaxation of the pseudo-cleavage furrow. Notably, this timing was sometime after the symmetry breaking, when the contractility of the cortex became asymmetric. I inhibited the cortical contraction and the formation of the pseudo-cleavage furrow by knocking down of nmy-2 and nop-1 genes, respectively, and found the EEM asymmetry was impaired. Based on the experiments, I concluded that the cortical contraction and the relaxation of the pseudo-cleavage furrow were critical for the EEM asymmetry.

In Chapter 3, I examined the shape and the movement of the P0 cell after removing the eggshell, to know the mechanism how the pseudo-cleavage furrow contributed to the EEM asymmetry. I found that the P0 cell migrated toward the posterior side upon the relaxation of the pseudo-cleavage furrow. In intact eggs, because this migration occurred inside the eggshell, the EEM was localized asymmetrically. From the observation of the shape of the P0 cell, I propose the mechanism of the migration of the P0 cell as follows. After the symmetry breaking, the contraction of the cell cortex leads to the constriction of the pseudo-cleavage furrow. This contraction elongates the cell along the long axis. Upon the relaxation of the furrow, the cell shortens along the long axis. This shortening occurs selectively at the anterior side because the contractility of the cortex is stronger at the anterior side, due to the abundant actin and myosin. This is a new mechanism for an adhesion-independent cell

migration, which will have general impact on cell migration study.

（別紙１ Separate Sheet 1）

Results of the doctoral thesis defense

博士論文審査結果

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Title
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多くの細胞は極性を持ち、様々な分子やオルガネラを非対称に局在させることが知られている。しかし、極性化により、細胞内に質量密度の偏りが生じるかは明らかではない。池田さんの所属研究室で、線虫 *C. elegans* の一細胞期卵を遠心顕微鏡（試料を遠心しながら観察できる顕微鏡）で観察したところ、卵の後極が遠心力方向に向くことを示唆する予備的な観察結果があった。池田さんは、この結果が卵に密度の偏りがあることを示していると考え、自身で遠心顕微鏡を用いてこの結果を確認した。次に密度を観察できる OI-DIC 顕微鏡で卵を観察したところ、細胞そのものには顕著な密度の偏りは見られず、その代わりに細胞と卵殻の間の extra-embryonic matrix (EEM)が細胞と比べて低密度であり、卵の前方に偏っていることを発見した。この EEM の非対称によって卵に密度の偏りが生じ、胚の向きが遠心力により決まると考えられた。次に EEM の非対称性が生じる過程を遠心顕微鏡で観察した。線虫胚は受精後の一細胞期に極性化し、前方に局在したアクトミオシンのはたらきで、細胞表層が収縮し、pseudocleavage と呼ばれる大きな溝が一過的に生じる。遠心力下では、pseudocleavage が解消されるまでに後極が遠心力方向へと配向した。また通常の培養条件での EEM も pseudocleavage の解消までに非対称になることが確認された。また、ミオシンの阻害や、pseudocleavage が起こらなくなる *nop-1* 遺伝子の阻害では EEM は非対称にならないことが分かった。以上の結果から、卵の前方でのアクトミオシンの収縮と pseudocleavage の解消により、EEM が非対称になることが示された。

非対称なアクトミオシンの収縮は移動する細胞で起こることがよく知られている。池田さんは、線虫の 1 細胞胚も卵殻内で僅かに移動する結果 EEM が非対称になると考えた。そこで、卵殻を除いた胚を観察すると、pseudocleavage が解消する際に前側の表層が特異的に縮む結果、細胞の中心が後側に移動することを発見した。さらにその後の細胞分裂の際、細胞の後側が伸長し、中心がさらに後側に移動した。一細胞胚は卵殻内で後向きに移動する力を発生していることが明らかになった。

細胞が基質などとの接着に依存せず移動する機構はいまだ明らかになっていない。池田さんが発見した卵殻除去胚の移動は接着に依存しないと考えられ、今後細胞移動研究のモデルとなる可能性がある。また卵殻と胚体との物理的相互作用は、二細胞期以降の適切な細胞配置に必須であり、EEM の非対称性もその後の発生に重要である可能性がある。以上のことから池田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。