博士論文の要約

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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.