

Graduate School of Sciences and Technology for Innovation,
Yamaguchi University

Division of Fundamental Sciences

A Computational Model of Cell Migration of Fish Keratocytes

Yu Tokunaga

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Chapter 1

Introduction

Amoeba proteus, a common ameba cell, migrates by stretching pseudopodia with changing its cell shape continuously. In contrast, fish keratocytes change their shape from a circular shape to a half-moon shape when they begin migration and keep its shape during migration. This phenomenon suggests that the deformation of the cell shape is a key feature to realize cell migration of keratocytes.

Actin proteins compose cytoskeleton of eukaryotic cells [1], and in keratocytes, actin molecules have been reported to extend their head toward the cell membrane by actin polymerization (AP), which has been suggested as a source of the deformation of the cell membrane and the propulsive force of the cell. The actin retrograde flow (ARF) that pulls the actin molecules back toward the stress fiber (SF), a bundle of actin fibers spreading from side to side of the rear part of the cell, has also been reported [2]. The purpose of this research is to clarify the mechanism that forms a half-moon shape by physical simulation experiments considering intracellular mechanism.

The structure of this paper is as follows. Chapter 2 introduces previous works on the molecular mechanism of cell migration of keratocytes. Chapter 3 explains the method of physical simulation experiments. Chapter 4 shows the simulation results, and the last chapter concludes this study and discusses the future prospects of this study.

Chapter 2

Keratocytes

2.1 Characteristics of Cell Migration of Keratocytes

A keratocyte, a migratory fish epidermal cell, is a wound healing cell about $70\text{ }\mu\text{m}$ in size. When a fish is injured, keratocytes begin migration toward the injured position toward the wound by the speed of about one body length in one minute. The locomotion of keratocytes is a kind of amoeboid movements; however, it is different from typical amoeba movement at the point that they move with keeping a half-moon shape.

The cell migration of keratocytes has been suggested to be caused by actin polymerization (AP)[1]. AP is a phenomenon in which cytoskeletal actin molecules linearly bind to form filamentous actin (F-actin). The AP occurs only at one end of F-actin, depolymerization occurs at the opposite end. The polymerized actin molecules form a dense network and push the cell membrane from the inside. It has been reported that the actin molecules are polymerized more frequently in higher density regions of actin molecules and depolymerized less in lower density regions [3]. There is a stress fiber (SF) at the rear of the cell. The SF is a bundle of actomyosin composed of actin molecules and myosin molecules. The actin retrograde flow (ARF) which pulls the actin molecules back toward the SF has also been reported [4]. Other study have reported that the ARF orient the actin molecules towards the SF[5]. When SF is removed, the shape of the cell collapses and the moving speed slows down. This also indicates the importance of SF for cell migration. Okimura et al. reported that the SF plays the role of wheels in cell migration [6]. It has been reported that the formation of a half-moon shape at the time of migration was observed even after removal of the nucleus [7]. These reports suggest that for the cell migration of keratinocytes, not the nuclear part but the other parts where SF and F-actin exist are important.

2.2 Molecular Mechanism of Cell Migration

A cell has a structure called a cytoskeleton. The cytoskeleton is like bones of humans and forms a cell shape, however, it shows dynamic behavior in contrast to the rigid nature of



Figure 2.1: Keratocytes during cell migration.(Source: Takako Tanaka, Iwadate Lab).

human bones. One of the main components of the cytoskeleton is actin molecules. The polymerization and depolymerization of actin molecules change the cell shape. The actin molecule extends toward the cell membrane by polymerization and is attracted to the SF by the ARF. The membrane of amoeboid cells is extruded by the polymerization of actin molecules and forms pseudopodia which adhere to the substrate under the pseudopodia. Thereafter, the adhesion to the substrates of the rear part of the cell is released and the cell is dragged forward. By repeating this cycle, cell migration is realized. The cell membrane of keratocytes is also deformed by actin molecule polymerization. By the simulation experiment described in the next chapter, we investigate how the cell membrane forms a half-moon shape by the actin molecule polymerization.

Chapter 3

Simulation Methods

3.1 Simulation Methods of Cell Membrane

In the computer simulation of this study, the cell membrane was modeled by a network of simple particles interacting with each other and placed on a cylindrical surface as an initial condition. Each particle of the membrane was assumed to receive elastic force from neighboring particles and repulsive force from actin molecules. The equation of motion of the cell membrane molecule was assumed as follows.

$$m \frac{d^2 \mathbf{x}_i}{dt^2} = \mathbf{F}_i^m + \mathbf{F}_i^a - \eta \frac{d\mathbf{x}_i}{dt} \quad (3.1)$$

where \mathbf{x}_i is the position vector of the membrane molecule, m is the mass of the particle, $\eta = 8.9 \times 10^{-6}$ kg/s is the viscous coefficient, \mathbf{F}_i^m and \mathbf{F}_i^a are the forces received from the neighboring particles and the repulsive force from actin molecules. The force \mathbf{F}_i^m was assumed as an elastic force:

$$\mathbf{F}_i^m = \sum_{j \in I_j} -k((\mathbf{x}_j - \mathbf{x}_i) - \mathbf{l}_{ij}) \quad (3.2)$$

where k is a spring constant, \mathbf{l}_{ij} is the natural length between the i -th and j -th membrane particles, and I_j represents the set of particles that interact with particle j . The natural length between particles was determined by the distance at the initial state. The force \mathbf{F}_i^a received from an actin particle was assumed to be a repulsive force:

$$\mathbf{F}_i^a = \sum_{\{\forall i \| \mathbf{x}_j - \mathbf{B}_i \| < D_2\}} \frac{s}{\| \mathbf{x}_j - \mathbf{B}_i \|} \frac{\mathbf{x}_j - \mathbf{B}_i}{\| \mathbf{x}_j - \mathbf{B}_i \|} \quad (3.3)$$

where s is a constant that determines the strength of the repulsive force, and D_2 is a distance range of the effect of the force prepared to decrease the calculation amount.

3.2 Simulation Methods of Actin Molecules

3.2.1 Actin Polymerization

An actin molecule has polarity: one end which elongates by the polymerization is called barbed-end, and the other end is called pointed-end. Since the frequency of polymerization is high in a region where actin molecules are dense [3], the polymerization rate was assumed to be proportional to the actin concentration. The filament formed by actin polymerization is called F-actin. F-actin was expressed by a simple rod in the simulation. As an initial condition, the length of each rod was zero and the direction of the actin polymerization \mathbf{L}_i was randomly determined. The elongation of the F-actin by the polymerization is modeled by

$$\mathbf{B}_i \leftarrow \mathbf{P}_i + f^p(c)\mathbf{L}_i \cdot dt \quad (3.4)$$

where \mathbf{B}_i and \mathbf{P}_i are the barbed end and pointed end of the F-actin, respectively, and $f^p(c) = 5.0 \exp \frac{c}{10.0}$ expresses the frequency of actin polymerization as a function of actin concentration c which was computed for each region by dividing the simulation space into a grid. The frequency of depolymerization decreases in an actin dense area and the depolymerization was expressed as follows.

$$\mathbf{P}_i \leftarrow \mathbf{P}_i + f^d(c)\mathbf{L}_i \cdot dt \quad (3.5)$$

where $f^d(c) = \frac{5.0}{c}$ represents the frequency of depolymerization. The actin polymerization and depolymerization was simulated by a probability

$$p_i(c) = c_i = \frac{a_i}{N}$$

at each step, where $p_i(c)$ is the probability that depends on the concentration c_i in i -th region, a_i is the number of actin in i -th region, and N is the total number of actin. In this simulation, the simulation space was divided into 225 regions ($i = 1, \dots, 225$).

The actin molecules in the region with a low density of actin molecules were assumed to disappear and the same number of new actin molecules were put near the cell membrane. The direction of polymerization of each actin was randomly determined at the initial state. In this experiment, the disappearance condition of the actin molecule is $c_i < 0.06$.

3.2.2 Actin Retrograde Flow

The ARF is a movement of F-actins toward the stress fiber which is a bundle of actin fibers aligning from side to side in the rear side of a keratocyte.

We assumed the ARF as the retraction of the actin molecules toward the leftmost point of the cell in Fig.3.2, i.e., the position of the membrane particle with the smallest x coordinate which we call the reference point. The retraction of the actin molecules by the ARF was

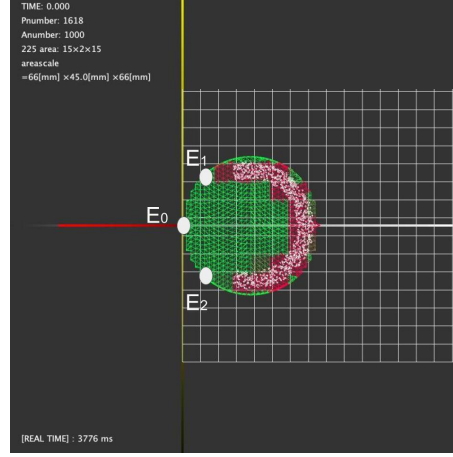


Figure 3.1: Location of the ARF reference point, \mathbf{E}_0 , \mathbf{E}_1 and \mathbf{E}_2 .

expressed by the following equation:

$$\begin{cases} \mathbf{B}_i \leftarrow \mathbf{B}_i - \alpha \frac{\mathbf{B}_i - \mathbf{E}_0}{\|\mathbf{B}_i - \mathbf{E}_0\|^w} \cdot dt \\ \mathbf{P}_i \leftarrow \mathbf{P}_i - \beta \frac{\mathbf{P}_i - \mathbf{E}_0}{\|\mathbf{P}_i - \mathbf{E}_0\|^w} \cdot dt \end{cases} \quad (3.6)$$

$$(3.7)$$

where \mathbf{E}_0 is the reference point, and α and β are constants that determine the strength of the ARF. w is a parameter indicating the effect of distance between the actin molecule and cell membrane molecule on the retraction strength of the ARF. When $w = 2$, the closer the intermolecular distance is, the stronger the retraction is. When $w = 1$, all actin molecules are equally pulled irrespective of the intermolecular distance. When $\alpha = \beta$, the orientation effect is ineffective, therefore, the actin molecule is retracted without changing the direction.

We also investigated the case that two reference points \mathbf{E}_1 and \mathbf{E}_2 are prepared in order to express the retraction of actin molecules toward the SF. In this case, assuming that SF spreads linearly at a distance of $1/5$ from the left end of the cell (Fig. 3.1) and the reference points \mathbf{E}_1 and \mathbf{E}_2 were put at both ends. The ARF was expressed by the retraction of actin molecules toward the direction of the sum of vectors from the actin molecules to the reference points:

$$\begin{cases} \mathbf{B}_i \leftarrow \mathbf{B}_i - \gamma \left(\frac{\mathbf{B}_i - \mathbf{E}_1}{\|\mathbf{B}_i - \mathbf{E}_1\|^w} + \frac{\mathbf{B}_i - \mathbf{E}_2}{\|\mathbf{B}_i - \mathbf{E}_2\|^w} \right) \cdot dt \\ \mathbf{P}_i \leftarrow \mathbf{P}_i - \delta \left(\frac{\mathbf{P}_i - \mathbf{E}_1}{\|\mathbf{P}_i - \mathbf{E}_1\|^w} + \frac{\mathbf{P}_i - \mathbf{E}_2}{\|\mathbf{P}_i - \mathbf{E}_2\|^w} \right) \cdot dt \end{cases} \quad (3.8)$$

$$(3.9)$$

where γ and δ are constants that determine the strength of the ARF. As in eq. 3.7, the orientation effect of ARF can be controlled by the magnitude relation of γ and δ . Table 3.1 shows the values of α, β, γ and δ .

Table 3.1: The value of each parameter.

	$w = 1$	$w = 2$	$w = 1$ (No orientation)	$w = 2$ (No orientation)
α	5.0	0.02	20.0	0.1
β	50.0	0.2	20.0	0.1
γ	2.5	0.01	10.0	0.05
δ	25.0	0.1	10.0	0.05

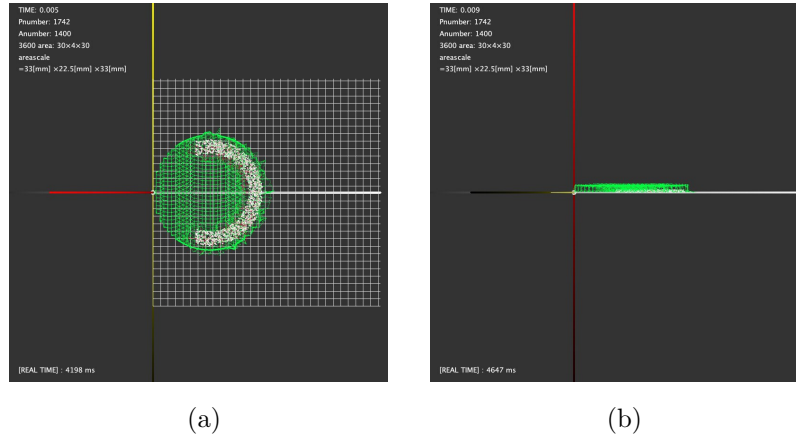


Figure 3.2: Initial state. (a) Top view. Green lines and white dots show actin molecules and membrane particles, respectively. (b) Side view.

3.3 Initial Condition

The number of actin molecules and membrane particles were 1,000 and 1,618, respectively, in the computer simulation. The positions of membrane particles were updated every step of the numerical computation and the positions of actin molecules were updated every 10 steps.

Fig. 3.2 shows the initial state of the virtual cell of the computer simulation. The actin molecule are in a U-shaped area excluding the rear of the cell (Fig. 3.2). The membrane molecules were placed on the surface of the cylinder.

Chapter 4

Results and Discussion

4.1 Simulation Results

4.1.1 Role of Actin Retrograde Flow

Fig. 4.1 shows the simulation result when two reference points of the ARF is prepared (eq. (3.9)) and the effect of the ARF is independent of the distance ($w=1$ in eq. (3.9)). In this case, the actin molecules aggregated in a half-moon shape and the shape was kept for a long time even after the time shown in Fig.4.1 (c).

Fig. 4.2 shows the results under the same condition as Fig. 4.1 except that distance-dependent ARF was assumed ($w=2$ in eq. (3.9)). Since actin molecules close to the SF are strongly pulled back in the case, actin molecules aggregated. As a result the AP, the region of actin molecule spreads elongate and repulsive force acts only on the front end of the cell membrane. The AP is activated in the region where actin molecule is concentrated and further extends beyond the cell membrane. When the repulsive force converges in this manner, the cell membrane is destroyed. Therefore, in the ARF which contracts the actin molecule polymerizing in the lateral direction, the crescent shape can not be formed.

Fig. 4.3 shows simulation results when one reference point of the ARF was prepared (eq. 3.7). The actin molecules migrated toward the center of the cell and the half-moon shape was not achieved. When the actin molecule aggregates in a circular shape, the direction of movement of the cell is not determined because it pushes the entire cell membrane. These results suggest that the retraction of actin molecules by the ARF toward not a point but the SF is an important phenomenon to make the cell shape the half moon.

Fig. 4.4 shows the simulation results when the retraction of actin molecules by the ARF was ignored. Actin molecules were continuously elongated by polymerization toward random directions. This result shows that the retraction by the ARF is important to keep specific cell size against actin polymerization.

Fig. 4.5 showed the simulation result when ignoring the orientation effect by the ARF.

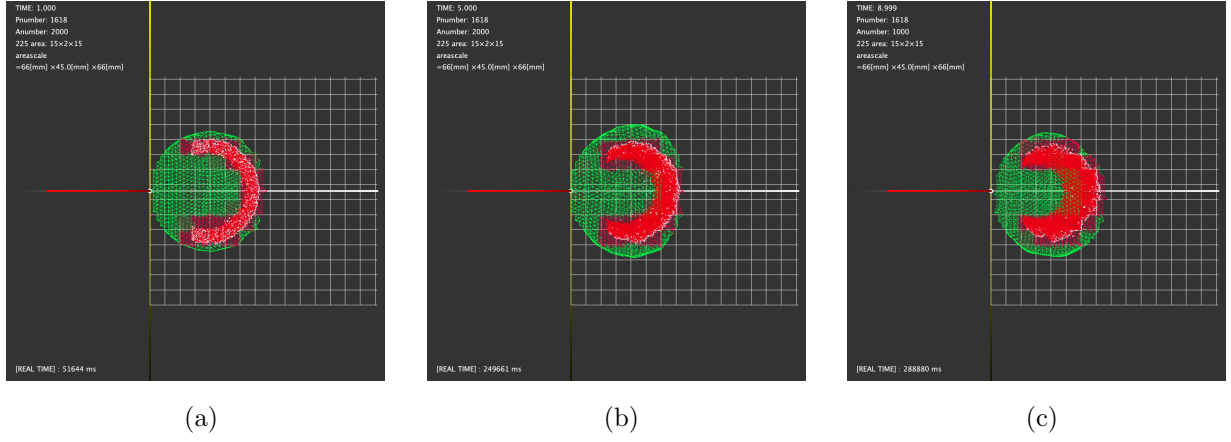


Figure 4.1: Simulation results when the amplitude of the retraction by the ARF is independent of the distance. (a), (b), and (c) show the results at $t = 1.0, 6.0, 11.0$ [s]. Green lines show the cell membrane, and white and red dots show the barbed-end of the F-actin, respectively.

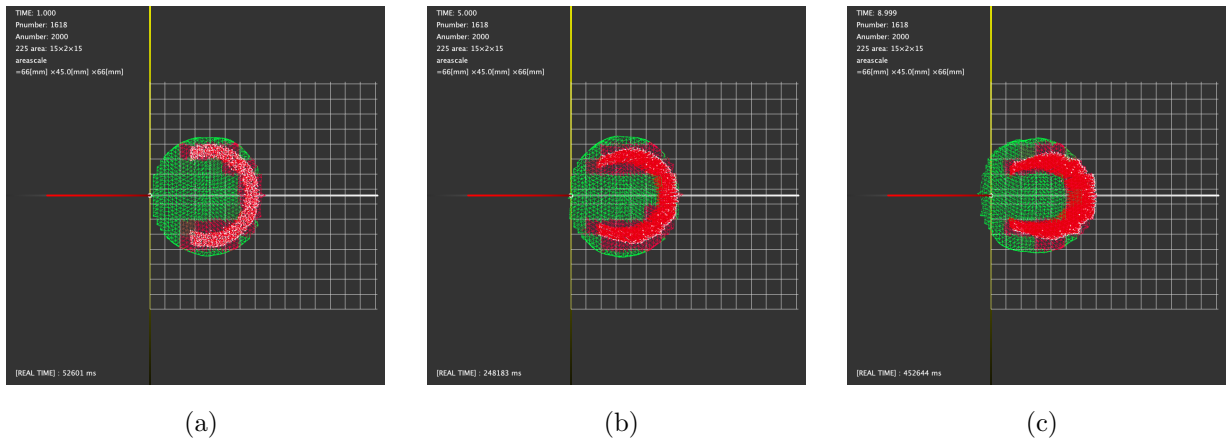


Figure 4.2: Simulation results under the same condition as those in Fig. 4.1 except $w = 2$ in eq. 3.9. The subfigures show the results at different timings as in Fig. 4.1.

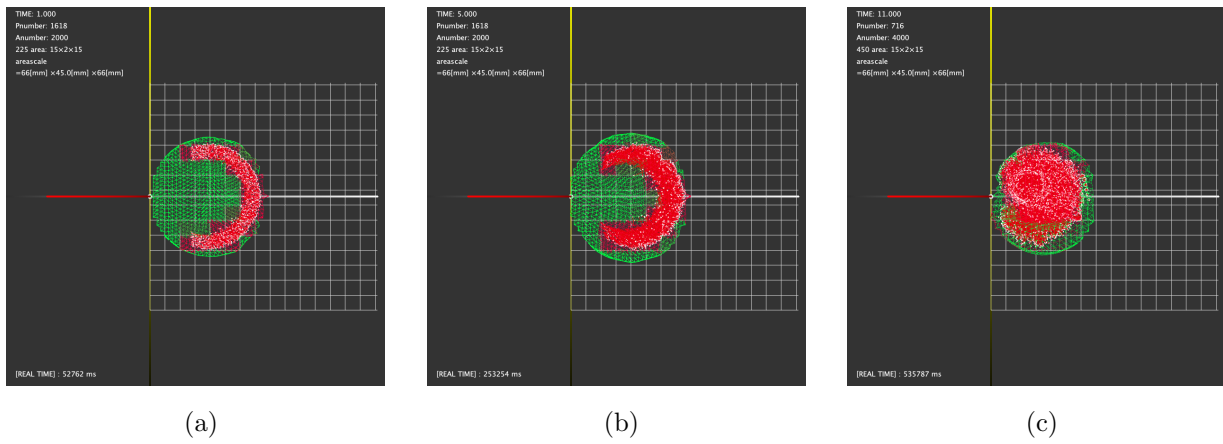


Figure 4.3: Simulation results when the reference point of the ARF was assumed at the position of the leftmost membrane molecule of the cell. The subfigures show the results at different timings as in Fig. 4.1.

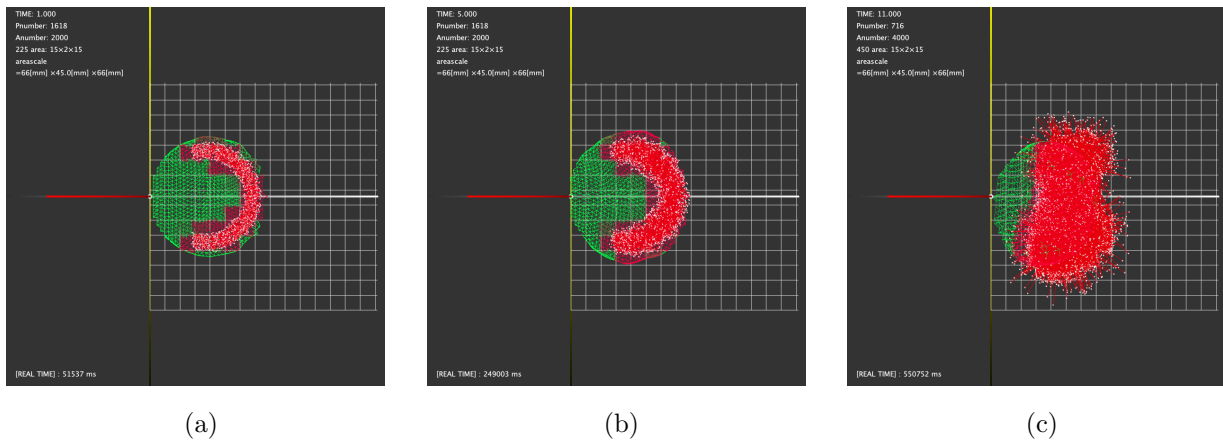


Figure 4.4: Simulation Results without ARF. The subfigures show the results at different timings as in Fig. 4.1.

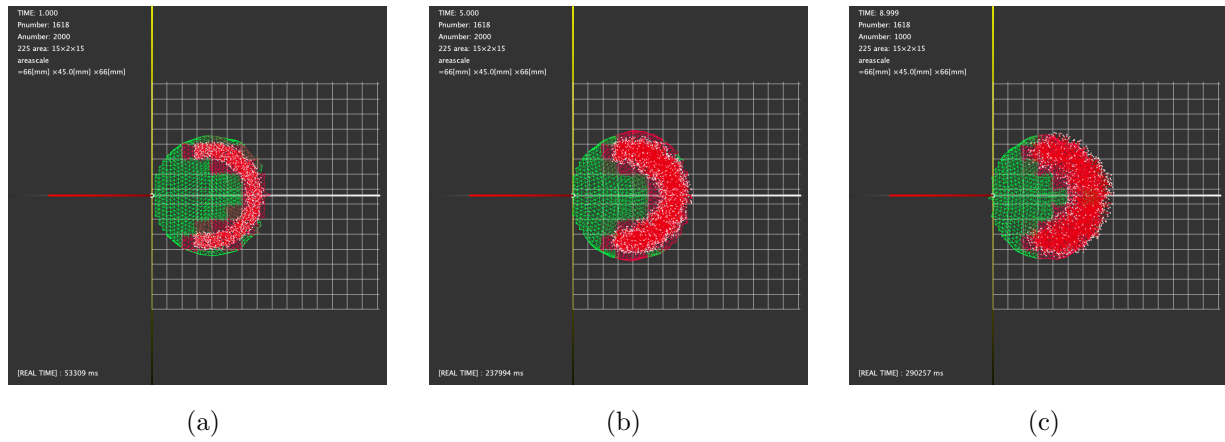


Figure 4.5: Simulation results without the orientation effect on the actin molecules by the ARF. The subfigures show the results at different timings as in Fig. 4.1.

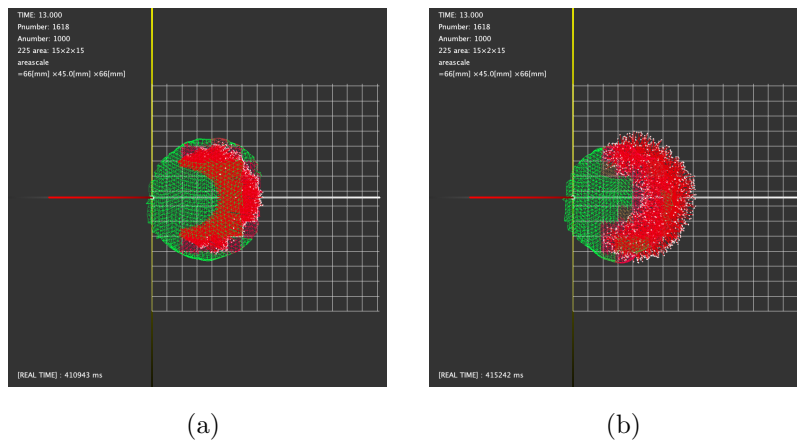


Figure 4.6: Simulation results at $t = 13$ [s]. (a) When there is orientation. (b) When there is no orientation.

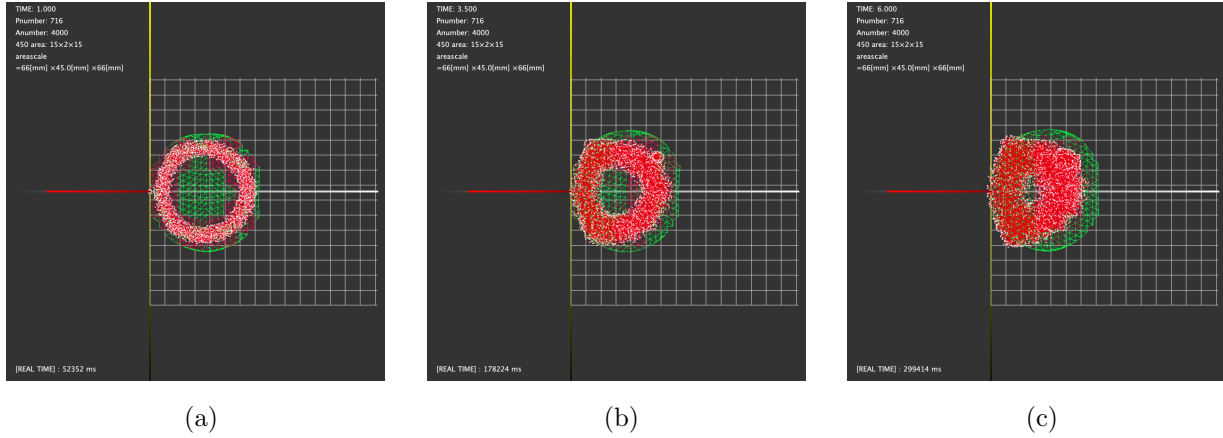


Figure 4.7: Simulation results when actin molecules are distributed in a circular region as an initial condition. The subfigures show the results at different timings as in Fig. 4.1.

The actin molecules aggregate into a V-shape and break the cell membrane. Paying attention to the white dots which is barbed-end, when compared with Fig.4.1, no alignment of the direction of the actin molecules was not observed. The shapes in Fig.4.5 and Fig.4.1 are similar, but since the barbed-end of the actin molecule is oriented in all directions, the shape when a certain time elapses differs (Fig.4.6). Without an orientation effect, the actin molecule region spreads without changing its shape. In contrast, in the presence of an orientation effect, the region of the actin molecule is within the cell membrane over time. This result suggests that the effect of aligning the polymerization direction prevents the expansion of the actin molecule region.

4.1.2 Effect of Initial Distribution of Actin Molecules

In the simulation experiments in the previous section, the actin molecules were randomly distributed in a U-shaped region as an initial condition. Fig. 4.6 shows the simulation results when actin molecules were distributed in a donut shape. The other simulation conditions were the same as those in Fig. 4.1. The actin molecule did not aggregate in a half-moon shape but around the SF. This is due to the presence of actin molecules already in the vicinity of SF when ARF acts with actin molecules arranged in a donut shape. From the results in Fig.4.1 and Fig.4.6, it is clear that the actin molecules aggregate in a U-shaped rather than a donut shape to form a half-moon shape. In keratocytes, the number of actin molecules decreases at the rear part of the cell when migration starts [8]. Hence, such a break of uniform distribution of the actin is a key to the formation of the half-moon shape.

4.1.3 Discussion on the Relationship between the AP and the ARF

All conditions under which the region of actin molecules aggregate in a half-moon shape are conditions related to the ARF. The AP in this simulation is only imposed to the condition

that the actin molecule extends in a specific direction and extends more in the region where the actin molecule is high. However, it was made clear in Fig.4.4 that actin molecules do not aggregate in a half-moon shape simply by simply polymerizing. The simulation results showed that the retraction and orientation of actin molecules by the ARF contribute to the formation of the half-moon shape and the regulation of the cell size, respectively.

When the actin molecule polymerizes so that it locally extends, it does not agglomerate in a half-moon shape. If all the actin molecules are not pulled uniformly, it is impossible to form a half-moon shaped circular arc as shown in Fig. 4.2. The effect of uniformly pulling the actin molecule contributes to the shape of the agglutinating region of the actin molecule as well as its maintenance.

Even when there is no orientation effect, if there is a retraction effect, actin molecules aggregate in a half-moon shape (Fig.4.5). However, in the absence of orientation effect, actin molecules repeatedly polymerize in all directions and the region of actin molecule continues to expand. The change in the direction of polymerization of actin molecule due to the orientation effect of the ARF is to adjust the barbed-end so that it extends radially from SF by aligning it in the direction of the SF. That is, the retraction effect is the effect of changing the position of the actin molecule, and the orientation effect is the effect of determining the next position of actin molecule. As the polymerization direction of actin molecule changes, the actin molecule density in each region also changes. As the actin molecule density in each region changes, the region where the actin molecule disappears and the region disappears change. As the area where actin molecule disappears increases, it becomes difficult to expand the agglutination size of actin molecule. It is speculated that ARF controls AP by such a mechanism.

Chapter 5

Conclusion

5.1 Conclusion

The purpose of this research is to clarify the mechanism that forms a half-moon shape by physical simulation experiments considering intracellular mechanism. We investigated how the cell membrane forms a half-moon shape when cytoskeletal actin molecule important for cell migration of migrating cells behave. In this experiment, the actin molecule which moves complicatedly by the AP and the ARF and the cell membrane which receives repulsive force from its actin molecule were modeled. As a result, the actin molecule aggregated in a half-moon shape due to the traction effect and orientation effect of the ARF. It was revealed that the ARF orientation effect contributes to keeping a half-moon shape and that both the ARF traction effect and the orientation effect contribute to the formation of a half-moon shape. These results show that ARF plays an important role in the formation of a half-moon shape.

5.2 Future Works

If the position of actin molecule is always maintained by the ARF, the cell does not move. More detailed conditions need to be added to the ARF condition. If the conditions of the ARF are constant depending on the location, but are not constant over time, another result may be obtained. There was a problem with the simulation of cell membranes receiving repulsive force from actin molecule. Even if the front end of the cell grew by the repulsive force of the AP, its effect did not propagate to the rear of the cell. In order to solve this problem, it is necessary to correct the binding relationship between the membrane molecules. If the conditions relating to the ARF can be improved and the cell membrane can be faithfully modeled as described above, the mechanism by which the cell membrane forms a half-moon can be elucidated.

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